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Strategies to address the long-term maintenance of bone mineral density in younger women

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Declaration of originality

This thesis contains no material which has been accepted for a degree or diploma by the University or any other institution, except by way of background information duly acknowledged in the thesis, and to the best of my knowledge and belief no material previously published or written by any other person except where due acknowledgement is made in the text of the thesis, nor does the thesis contain any material that infringes copyright.

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"The research associated with this thesis abides by the international and Australian codes on human experimentation, and the rulings of the Safety, Ethics and Institutional Biosafety Committees of the University."

The Human Research ethics Committee (Tasmania) Network approved this project (Approval number H11613: Strategies to address the long term maintenance of bone density in younger women: fracture risk feedback and vitamin D). We obtained written informed consent from all participants.

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Statement of authorship

This thesis includes papers for which Feitong Wu (FW) was not the sole author. FW was the first author in the research of each manuscript; however, he was assisted by the co-authors whose contributions are detailed below.

Chapter 4

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FW analysed the data, wrote the draft manuscript and completed revisions.

KW assisted with data analysis and provided statistical advice.

TW, GJ and BO designed this study and formulated the hypotheses for this analysis.

All authors contributed to data interpretation, critically revised the manuscript for important intellectual content, and read and approved the final manuscript.

Chapter 5

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KW assisted with data analysis and provided statistical advice.

TW and GJ designed this study and formulated the hypotheses for this analysis.

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Chapter 7

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KW and TW assisted with data analysis and provided statistical advice.

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Chapter 8

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FW analysed the data, wrote the draft manuscript and completed revisions.

KW and MC assisted with data analysis and provided statistical advice.

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Abstract

Long-term maintenance of bone mineral density (BMD) in premenopausal women is critical to preventing osteoporosis and osteoporotic fractures. This thesis aimed to investigate potential strategies to optimise long-term bone health in younger women in a cohort of women who 12 years previously had participated in a 2-year randomised controlled trial (RCT) of an osteoporosis education intervention. In the original trial, women were randomised at baseline to receive group education (the Osteoporosis Prevention and Self-management course (OPSMC)) or an information leaflet. All women also received individualised feedback of either being or not being at higher risk of fracture in later life (high and normal risk groups). The risk was based on BMD measured by Dual-energy X-ray absorptiometry (DXA) according to whether or not each participant's mean T-score at spine and hip was less than 0 (high and normal risk groups, respectively).

For this thesis, we performed a further 10-year follow-up, i.e. 12 years from baseline of the original RCT. We measured osteoporosis knowledge, self-efficacy, BMD at the femoral neck (FN) and lumbar spine (LS); calcium intake and calcium supplement use, physical activity and smoking status as in the original RCT. In addition, we measured serum 25-hydroxyvitamin D (25(OH)D), lower limb muscle strength (LMS), timed up and go test (TUG), functional reach test (FRT), lateral reach test (LRT) and step test (ST); total physical activity (accelerometer counts/minute of wear time), and time spent sedentary, in light and moderate-to-vigorous physical activity (MVPA).

Longitudinal data were used to investigate the long-term effects for the RCT interventions on BMD and osteoporosis preventive behaviours. Cross-sectional data were used to examine associations between the modifiable factors of vitamin D levels, LMS and physical activity and BMD and balance measures.

Key findings were:

Longitudinal data:

1. From baseline to 12 years, neither feedback of high fracture risk nor the OPSMC had an effect on the change in osteoporosis knowledge or self-efficacy.
2. From 2 to 12 years, the high fracture risk group had a smaller decrease in FN BMD (β=0.023 (95% CI: 0.005-0.042) g/cm^2) but similar LS BMD change as the normal risk group. They also had a more favourable pattern of smoking behaviour change and were more likely to use calcium supplements and be recent users of vitamin D supplements. The OPSMC group had a more favourable pattern of smoking behaviour change compared to the leaflet group.

Cross-sectional data at 12 years:

3. There were significant cut-points for associations of 25(OH)D levels with FN BMD, LS BMD, TUG, ST, FRT and LMS (ranging from 29-33 nmol/L) but not LRT. Below these cut-points, there were beneficial associations between higher 25(OH)D level and each outcome while above the cut-points there were no beneficial associations.

4. Weaker LMS was associated with poorer performance on all balance tests. Significant cut-points of LMS were identified for all balance tests (29-50 kg) but excepting ST, these did not persist after excluding potentially influential data points.
5. Total physical activity was beneficially associated with FN BMD, LMS and TUG. MVPA was also beneficially associated with FN BMD, LMS, ST and TUG, and these associations (except for FN BMD) persisted after further adjusting for sedentary time. Sedentary time was detrimentally associated with TUG but not after further adjustment for MVPA.

In conclusion, feedback of high fracture risk to younger women was associated with long-term improvements in osteoporosis preventive behaviours and attenuated FN BMD loss and could be considered as a strategy to improve long-term bone health and prevent osteoporosis. Furthermore, we identified other potential strategies for maintaining BMD and balance in middle-aged women, namely:

- Maintaining adequate serum 25(OH)D noting that the current cut-off defining vitamin D deficiency of 50 nmol/L may be higher than needed for some musculoskeletal outcomes but appears warranted overall.
- Improving LMS.
- Increasing time spent in MVPA.

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List of publications

Publications arising from this thesis:

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Wu F, Callisaya M, Laslett LL, et al. Lower limb muscle strength is associated with poor balance in middle-aged women: linear and nonlinear analyses. Osteoporosis International. 2016:27:2241-8.

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Scientific presentations and awards

2016 IOF Regionals – 6th Asia-Pacific Osteoporosis Meeting Singapore (oral presentation + IOF Young Investigator Award with 1,000 USD honorarium);

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2015 Australian and New Zealand Bone and Mineral Society (ANZBMS) annual meeting, Hobart, Australia (three poster presentations);

2014 Nutrition Society of Australia (NSA) annual meeting (oral presentation);

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2014 Australian and New Zealand Bone and Mineral Society (ANZBMS) annual meeting, Queenstown, New Zealand (poster presentation and travel grant);

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List of abbreviations

25(OH)D 25-hydroxyvitamin D

BMI Body mass index

BMC Bone mineral content

BMD Bone mineral density

CCT Clinical controlled trial

DALYs disability-adjusted life years

DXA Dual energy X-ray absorptiometry

FFQ Food frequency questionnaire

FN femoral neck

FRT functional reach test

ICC Intraclass correlation coefficient

IOM the Institute of Medicine

LMS lower limb muscle strength

LRT lateral reach test

LS lumbar spine

MVPA moderate-to-vigorous physical activity

OKAT Osteoporosis knowledge assessment tool

OPSMC Osteoporosis prevention and self-management course

OR Odds ratio

RDI Recommended dietary intake

RR Relative risk

RCT Randomised controlled trial

SD Standard deviation

ST step test

TUG timed up and go test

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Chapter 1: Literature review

1.1 The importance of osteoporosis and associated fractures

Osteoporosis is a progressive bone disorder characterised by low bone mass, micro-architectural deterioration of bone tissue, and skeletal fragility, leading to reduced bone strength and increased susceptibility to fracture. Bone strength primarily reflects the integration of bone density and bone quality[1]. Given the difficulty in measuring bone strength, bone mass has long been recognized as the most useful surrogate for strength, although they are not perfectly correlated[2]. In the absence of a fragility fracture, bone mineral density (BMD, measured by dual-energy x-ray absorptiometry (DXA)) is used for the diagnosis of osteoporosis according to the World Health organization (WHO), which has defined osteopenia (also known as low bone mass, *T*-score -1 to -2.5) and osteoporosis (*T*-score < -2.5) based upon T-score, that is, the number of standard deviations by which an individual's BMD deviates from the mean BMD in healthy young adults of the same gender and ethnicity[3].

Osteoporosis is a major public health issue, currently affecting over 200 million people worldwide[4]. The number of affected persons continues to increase as the global population ages. Importantly, osteoporosis in many cases remains "silent" and asymptomatic until a fracture occurs: Fragility fractures (also known as osteoporotic fractures or low-trauma fractures), which occur from a fall from a standing height or less, with no or low trauma, are the most important clinical consequence of osteoporosis. These fractures are common throughout the world. It was estimated that nine million osteoporotic fractures occurred worldwide in 2000, of which 1.6 million were at the hip, 1.7 million at the forearm, and 1.4

million were clinical vertebral fractures[5]. In Australia, a recent report launched by Osteoporosis Australia indicated that 4.74 million people aged over 50 years (66%) have osteoporosis or osteopenia (low bone mass, T-score -1 to -2.5), and this number is estimated to increase to 6.2 million by 2022[6]. Older adults have a very high estimated residual lifetime fracture risk. In the Dubbo Osteoporosis Epidemiology Study (Australia), it was estimated a residual lifetime risk of 29% for men and 56% for women aged 60 years and older[7]. The report also indicated that there were 140,822 fractures arising from osteoporosis or osteopenia in 2012, and this will increase to over 183,105 fractures by 2022[6]. In the United States, it has been estimated 54 million adults aged 50 and older were affected by osteoporosis or osteopenia in 2010[8] and this number will increase significantly to an estimate of 64 million by 2021 and 71 million by 2031[8]. According to the 2004 Surgeon General's Report on Bone Health and Osteoporosis, almost 40% of White American women aged 50 or older is expected to experience a hip, spine, or wrist fracture during the remainder of their lives.

The consequences of fractures are substantial in both the short and long term. These include increased risk of mortality and disability, and decreased quality of life. Sustaining one fracture also increases the risk of subsequent fracture: a meta-analysis of 11 cohort studies (15259 men and 44902 women) demonstrated that individuals who previously suffered from a fracture had significantly increased risk of any fracture compared with those who did not (relative risk (RR) = 1.86; 95% confidence interval (CI) = 1.75-1.98)[9]. A systematic review of 22 studies showed that individuals with hip fractures have a significant excess risk for mortality of at least double that of the age-matched controls (the smallest risk among the included studies); this excess risk persists for several years after the index fracture, although no meta-analysis or pooled analysis were conducted due to lack of consistency in the study

designs and statistical analyses across studies[10]. In 2000, the total global loss of Disability Adjusted Life Years (DALYs) was 5.8 million, of which 51% were accounted for by fractures that occurred in Europe and the Americas[5].

The financial implications of osteoporosis and fractures is also of concern, both for governments and the community. In Australia the total health expenditure for osteoporosis and osteopenia in individuals over 50 years of age was $2.75 billion in 2012 and it is predicted that this will increase to $3.84 billion in 2022[6]. It is predicted that total direct and indirect cost of osteoporosis, osteopenia and consequent fractures will be $33.6 billion from 2012 to 2022[6]. It is estimated that annual costs of all osteoporotic fractures are around $20 billion for the United States and $30 billion for Europe[11,12].

1.2 Peak bone mass and age-related bone loss

1.2.1 Peak bone mass acquisition

Peak bone mass refers to the amount of bony tissue present at the end of the skeletal maturation[13]. While the exact age at which peak bone mass is achieved varies with genetic, hormonal, and environmental factors and to skeletal site and method by which BMD is measured, the acquisition of bone mass persists in most individuals until the third decade of life, with up to 90 percent of peak bone mass achieved by age 18 in girls[14] and by age 20 in boys[15].

1.2.2 Maintenance of bone mass is important for the prevention of fractures

BMD in later life is determined by peak bone mass achieved by an individual and the rate of subsequent bone loss[13], with low BMD independently predicting fractures in later life[12]. In

a meta-analysis of 11 prospective studies, for one standard deviation (SD) decrease in femoral BMD below age adjusted mean, there was a 2.6-fold increase in relative risk of hip fracture[16]. Therefore, the main strategies for preventing low bone mass in older adulthood are to maximize peak bone mass and minimize the rate of bone loss, and ultimately to maintain bone strength and prevent osteoporosis and fractures.

1.2.3 Premenopausal bone loss

Longitudinal data suggest that age-related bone loss begins prior to the onset of menopause[17-21], although the magnitude of this loss varies, including by bone site. The exact time at which this decline begins is uncertain, but it is most likely to occur at approximately 30 years of age[18,22], and persists at a slow rate until menopause. A 6-yr prospective study reported an annual loss of 0.3% at the femoral neck BMD in a cohort of 614 women aged 24-44 years at baseline[20]. Similarly, longitudinal data have shown a decrease of femoral neck bone mineral content (BMC) at a rate of 0.22% per year and BMD at a rate of 0.43% per year in 130 healthy premenopausal white women aged 31-50 years while there was significant increase of the spine (L2-L4) BMD (0.19% per year) and BMC (0.41% per year) but no significant change of these measures at the total hip[21]. This might be partly due to the site specific response of BMD to lifestyle and environmental factors, for example, exercise[23]. Also, age-related bone loss may also be site-specific as the age of attaining peak bone mass may differ by site of bone measured[24].

This slow rate of change is important, as the cumulative bone loss from age 30 until menopause (at around 50 years old) is substantial. For example, there would be a decline of about 0.5 SD from peak bone mass during a period of 20 years even if the annual loss is only 0.2%.

1.2.4 Menopause transition and bone mass

Longitudinal studies have documented a substantial decline in bone mass in women in the perimenopausal[22,25] and newly post-menopausal periods[26], in data from different cohorts. Seifert-Klauss et al. showed acceleration of bone loss at the lumbar spine (measured by quantitative computer tomography) during perimenopause, reaching > 50% of the maximal total bone loss measured around menopause, regardless of adequate serum estradiol levels[25]. In a cohort of 75 women aged > 46 years having premenopausal estradiol and gonadotropin levels and regular menses, a sigmoid pattern of bone loss was observed across menopause in those who experienced normal menopause during 9.5 years of follow-up, beginning around 2-3 years prior to the last menses and ending around 3-4 years after the last menses[26]. The total estrogen-deprivation bone losses were 10.50, 7.73, and 5.30% for the spine, total body, and femoral neck, respectively. In a larger cohort of 1902 multiethnic women aged 42-52 years at baseline with an average follow-up of 3.9 years, little change occurred in lumbar spine or total hip BMD during the pre- or early perimenopause (menopause stage was determined based on reports about frequency and regularity of menstrual bleeding at each annual visit)[22]. However, BMD declined substantially in the late perimenopause, with an average loss of 1.6% per year in the lumbar spine and 1.0% per year in the total hip, while in postmenopausal women, there was an even larger bone loss at an annual rate of 2.0% and 1.4% at lumbar spine and total hip, respectively[22].

1.2.5 Tracking of BMD in adult women

Although a number of studies have been conducted on the tracking of BMD in children and adolescents[27-30], this has rarely been investigated in adults and has not been studied exclusively in female participants. In younger adults, Emaus et al. illustrated that there was a

high degree of tracking of BMD at both distal and ultradistal forearm sites for both men and women aged 25-44 years over an average follow-up period of 6.4 years (as assessed by Pearson's correlation coefficient, > 0.93 for both sites and both genders)[31]. When BMD values measured at baseline and at follow-up were divided into quartiles, 75-80 percent remained in the same quartile position from baseline to follow-up. Ten to thirteen percent either lost or gained only one position at the distal forearm site and these individuals were evenly distributed across all original quartile positions[31]. Another study by Emaus et al. showed similarly high correlation between BMD (distal and ultradistal forearm) at baseline and end of an average follow-up period of 6.5 years among Norwegian women and men aged 45-84 years (r > 0.90 in women and > 0.93 in men), and less than 30% relocated in the quartile positions, suggesting a high degree of tracking of bone mineral density measurements[32]. These findings suggest that adults with low BMD in younger adulthood are likely to also have low BMD in later life in the absence of any intervention(s), meaning that young adults with low BMD are at higher risk of osteoporosis and fractures in older age, compared to young adults with normal BMD. However, this needs to be confirmed by direct evidence assessing the association of BMD in early life with fractures in later life.

1.3 Muscle strength, balance and osteoporotic fracture

1.3.1 Relationship between muscle strength and balance in younger adults

Muscle strength is a major factor in balance, gait and postural stability[33-35]. Many studies have shown that age-related loss of muscle strength is an important contributor to decreased balance and functional limitations in older people[33-36], but studies are limited in younger adults[37-41]. In a cross-sectional study of young healthy adults, no statistically significant associations were found between balance measures (displacements of the center of pressure

in anterior-posterior/mediolateral direction under dynamic/static conditions) and isometric muscle strength (r ranged from +0.041 to +0.387, $p > 0.05$ for all)[37]. This may partly be explained by the small sample size (n = 27) and very young age (mean age (SD) = 23 (4) years), when it could be assumed most people have fairly good physical condition and muscle strength is above the threshold required to maintain balance. In contrast, one cross-sectional study in 1346 middled-aged women (age = 53 years for all participants) reported that greater grip strength was associated with better chair rise performance and the ability to balance on one leg with eyes open for 5 seconds[39]. Analysis of baseline data in a pre-post study of a muscle strengthening intervention in 26 middle-aged women (mean age 52.8 (SD 2.4) years) reported that greater maximal isometric bilateral leg extension force was moderately associated with better performance in the test of "10-m walk time" at baseline ($r = -0.6$, $p<0.01$), though no effects on static balance or time of standing on 1 leg or climbing for 10 steps were observed[40]. This may be explained by the small sample size, fairly good physical condition (people with severe diseases or musculoskeletal problems or contraindications to exercise were excluded) and moderate muscle strength of study participants before intervention. In contrast, one small cross-sectional study did not identify any associations between balance and LMS measures in middle-aged adults (n=32 of whom only 9 were female, mean age 56 (SD 4) years)[38], probably due to the very small sample size[38]. Longitudinal data in healthy middle-aged men (45-68 years old) has shown that those in the lowest and middle tertile of baseline grip strength were at greater risk of developing functional limitations and disabilities than those in the highest tertile (risk ratio (RR) = 1.07-2.80 for a range of measurements)[41].

1.3.2 Adequate muscle strength and balance are critical for the prevention of fractures

Falls increase the risk of hip fractures[42,43], while muscle weakness (especially of the lower extremities)[43,44] and impaired balance increases risk of falls[43,45], with 4-39% of falls in people older than 65 years attributed to gait/balance disorders[46]. It is possible to improve muscle strength by exercise programs: a randomised controlled trial of 2-yr progressive, resistive back-strengthening exercise program in postmenopausal women (n = 50, aged 58-75 years) significantly improved back extensor strength while reducing the risk of vertebral compression fracture by 63%, compared to a no intervention control group[47]. Therefore, muscle weakness and impaired balance may plausibly mediate fracture risk through greater susceptibility to falls.

Importantly, muscle strength and balance decline as people age, with muscle strength declining by 1.5% between ages 50 and 60 and by 3% thereafter[48] and balance declining after 45-55 years of age[49,50], particularly in women. Therefore, prevention of functional limitations, falls and fractures in older age via maintaining adequate muscle strength and balance may need to begin in early midlife. However, more clinical trials are needed to confirm whether improving muscle strength in younger middle life is effective at maintaining balance and preventing falls and fractures in later life, and whether maintaining such improved strength is necessary for functional improvements to persist.

1.4 Modifiable lifestyle factors for the prevention of osteoporosis

There are a range of potentially modifiable risk factors affecting BMD, such as poor nutrition, being physically inactive, and other lifestyle measures (e.g., alcohol consumption

and eating disorders, etc.). This chapter will focus on four key risk factors, namely, low calcium intake, vitamin D deficiency, smoking and being physical inactive.

1.4.1 Calcium and vitamin D

1.4.1.1 The impacts of calcium and vitamin D on bone health in younger women

There is substantial evidence that sufficient intakes of calcium and adequate vitamin D levels are needed to maintain bone mass and to protect against osteoporosis and fractures. The importance of vitamin D for bone health is mainly attributed to its role in calcium homeostasis and bone metabolism. Several meta-analysis of RCTs have shown a beneficial reduction in fractures with supplementation of calcium alone[51,52] or calcium plus vitamin D[52-54] but not vitamin D alone[55-57], compared to placebo or no treatment in older women. However, evidence is unclear in premenopausal women due to an evidence gap: the 2013 United States Preventive Services Task Force concluded that there was insufficient evidence to assess the benefits and harms of combined calcium and vitamin D supplementation for the primary prevention of fractures in premenopausal women[58]. Nevertheless, a meta-analysis of RCTs showed that daily calcium supplementation of 1000 mg reduced bone loss by 1% annually at all bone sites except the ulna amongst premenopausal women[59].

Similarly, despite the lack of clinical evidence on the efficacy of vitamin D supplementation for reducing fractures in younger women, Di Daniele and colleagues showed that supplementation of vitamin D plus calcium had beneficial effects on total body BMD in both peri- and post-menopausal women[60]. Moreover, low vitamin D status is associated with low spine BMD and increased bone turnover[61], which is concerning as vitamin D deficiency (serum 25(OH)D concentrations < 50 nmol/L) is very common in young women (more

details in Chapter 1.4.1.5). However, a 2-yr double-blind crossover study failed to show beneficial effect of 800 IU vitamin D supplementation daily on BMD or bone turnover markers in 70 pre- and post-menopausal women aged 24-70 years (mean age = 47.2 years)[62]. This may be explained by the high baseline serum 25(OH)D (mean = 68 and 76 nmol/L for the treatment and control group, respectively), but low daily dietary calcium intake (553 and 586 mg) and the broad age range of study participants.

Overall while adequate calcium and vitamin D are currently recommended as essential components of good nutrition for the prevention of osteoporosis in younger women, the evidence base supporting this is limited.

1.4.1.2 The impacts of vitamin D on muscle strength and balance in younger women

Muscle strength

Most observational studies reporting associations between serum 25(OH)D and muscle strength were conducted in older adults; however, a few were conducted in younger women[63-65]. A recent cross-sectional study of 137 younger women (age range 19-29 years) demonstrated a small but significant association between plasma 25(OH)D and handgrip strength (β = 0.05 (0.01-0.09) and 0.04 (0.001-0.08) for dominant and non-dominant hand)[64]. Grimaldi et al. reported a positive association between serum 25(OH)D and both isometric and isokinetic arm strength as well as isometric but not isokinetic leg strength among 419 healthy men and women over a broad age range (20-76 yr)[65]. However, interactions between serum 25(OH)D and gender or age were not assessed in the analyses, thus the actual relationship between serum 25(OH)D and muscle strength in younger women was unknown. In contrast, Marantes et al. showed there was only a weak relationship

between serum 25(OH)D and skeletal muscle mass but not muscle strength (isometric knee extension moment) in a subgroup analysis of women aged 21-65 years[63]. The discrepancies between these two studies could be partly explained by differences in the age of study participants and parameters measured for muscle strength.

Two recent meta-analyses of RCTs (17 studies involving 5072 participants[64] and 30 studies involving 5615 participants[65], respectively) have consistently shown a beneficial effect of vitamin D supplementation on various measures of muscle strength in men and women (most included studies were in older adults) with low serum 25(OH)D (below 25-30 nmol/L). However, there are only a few RCTs examining the effects of vitamin D with or without calcium supplementation on muscle strength in younger women[66-68]. A small RCT among 40 healthy young vitamin D-deficient Asian Indians (baseline serum 25(OH)D < 50 nmol/L for all participants, 24 males/16 females, mean age = 31.5 years) showed that six months of oral vitamin D and calcium supplementation (60 000 IU cholecalciferol per week for 8 weeks followed by 60 000 IU/month for 4 months, with 1 g of calcium daily) improved skeletal muscle strength (handgrip strength and gastro-soleus strength) as compared with placebo controls[68]. However, a similar RCT of larger sample size (n = 173) of young Asian Indian women demonstrated that vitamin D and calcium supplementation (60,000 IU cholecalciferol/wk for 8 weeks followed by 60,000 IU/fortnight, with 500 mg calcium twice per day for 6 months) did not lead to an improvement in muscle strength (hand grip and pinch grip strength)[67]. In support of this, a more recent study showed that 16-weeks of daily supplementation with 1000 IU vitamin D3 (cholecalciferol) or 400 IU vitamin D3 did not improve muscle strength or power among 251 healthy adult males and females from ethnic minorities in Norway (age range 18-50 years), compared to placebo, despite low baseline vitamin D status (mean = 26 nmol/L, 92% less than 50 nmol/L)[66]. These findings are limited

by small sample sizes and short periods of supplementation, as well as differences in frequency and dosages of supplement and parameters used to measure muscle strength. Therefore, larger RCTs of longer duration of follow-up using validated and reproducible measurements of muscle function are needed to provide compelling evidence for clinical recommendations, particularly in younger people.

Balance

Although associations between 25(OH)D levels and balance have been described in a number of cross-sectional studies among younger women[69-75], no consensus has been reached and this could be partly explained by the high heterogeneity of parameters used to assess balance, such as 8-ft walk, 6-min walk, gait speed, and sway.

Two small RCTs have been conducted in younger women[68,76]. In the RCT described above, Gupta and colleagues demonstrated that six months of cholecalciferol (60 000 IU D_3/week for 8 weeks followed by 60 000 IU/month for 4 months) and calcium (1 g of elemental calcium daily) supplementation led to enhanced performance on walking distance compared to those who received dual placebos[68]. In contrast, a recent RCT among 130 non-western overweight men and women (aged 20-65 years) in Netherlands who had baseline 25(OH)D level <= 50 nmol/L, showed that four-months of daily supplementation with 1200 IU vitamin D_3 and 500 mg calcium did not have an effect on physical performance (physical performance score, determined as the sum score of walking test, chair stand test and tandem stand) or exercise capacity (6-min walk test) in the intention to treat analysis, compared to the control group who received placebo and 500 mg calcium[76]. However, a *post hoc* analysis among participants who reached a serum 25(OH)D concentration of > 60 nmol/l after intervention, demonstrated an improvement of 19 m in the 6-min walk test compared with the

control group (p = 0.053). The minimum clinically relevant change for the 6-minutes walking test was 30 m in older patients with heart failure[77], but the clinical importance of an improvement of 19 m (about 0.26 SD) in overweight adults remains unclear. The discrepancy between studies could be mainly explained by significantly different dose of vitamin D (> 8500 IU/d on average vs. 1200 IU/d) supplementation and different outcomes used. Although compliance with supplementation might also contribute to the difference, it is unlikely to be the case for these two studies given their fairly good compliance (an average of >80% intake of the prescribed pills vs. 95% took all tablets) and large differences in the doses administrated. Therefore, these findings should be confirmed by larger RCTs of high vitamin D supplement dose using validated and reproducible measurements of balance.

1.4.1.3 Optimal serum 25(OH)D levels

People can obtain vitamin D in two ways, both via the action of sunlight on skin (D3 or cholecalciferol) and from a limited range of foods. Currently, based on serum 25(OH)D, the optimal indicator of vitamin D status[78], the optimal vitamin D status has been established based primarily on clinical evidence for skeletal health, but the value remains controversial. It is generally agreed that 25(OH)D levels less than 50 nmol/L are suboptimal for skeletal health[79]. In comparison, clinical data for extraskeletal health is scarce, hampering the efforts of establishing the optimal level in the context of benefiting a broad spectrum of health conditions.

1.4.1.4 Recommended daily intakes of calcium and vitamin D in young and middle-aged women

The Institute of Medicine (IOM) recommends a daily intake of 1000 mg of calcium is needed for women aged 19-50 years and 1200 mg/day for those older than 50 years (**Table 1.1**)[78]. In Australia, the same amount of calcium is recommended for women aged 19-50 years but slightly higher for those older than 50 years, that is, 1300 mg/day (RDI in **Table 1.2**)[80].

The recommended intake of vitamin D is normally established based on the assumption of minimal or no sun exposure. The IOM calls for 600 IU of vitamin D daily for all ages up to age 70, corresponding to a serum 25(OH)D level of at least 20 ng/ml (50 nmol/L) (**Table 1.1**)[78]. According to the IOM, this level is sufficient to meet the requirements of at least 97.5% of the population, based primarily on bone health. In Australia, the adequate intake (AI) is 200 IU/d for women aged 19-50 years and 400 IU/d for those aged 50-70 years (**Table 1.2**). It should be noticed that the AI for younger women (19-50 years) is based on the amount of vitamin D required to maintain a serum 25(OH)D level of at least 27.5 nmol/L with minimal exposure to sunlight. Therefore, the AI should be much higher when a serum 25(OH)D level of at least 50 nmol/L has to be achieved.

Table 1.1: Calcium and vitamin D dietary reference intakes in women aged 19-70 years

Age group	Calcium	Vitamin D	
	RDA (mg/day)	RDA (IU/day)	Serum 25(OH)D level (ng/ml) (corresponding to the RDA)[a]
19 to 30 years old	1000	600	20
31 to 50 years old	1000	600	20
51 to 70 year old	1200	600	20
Pregnant/lactating			
19 to 50 years old	1000	600	20

RDA, recommended dietary allowance;
[a]Measures of serum 25(OH)D levels corresponding to the RDA and covering the requirements of at least 97.5% of the population.

Table 1.2: Australian recommended dietary intake for calcium and vitamin D intake in women aged 19-70 years

Age	Calcium		Vitamin D
	EAR	RDI	AI[a]
19-30 yr	840 mg/day	1,000 mg/day	200 IU/day
31-50 yr	840 mg/day	1,000 mg/day	200 IU /day
51-70 yr	1,100 mg/day	1,300 mg/day	400 IU /day

EAR, estimated average requirement; RDI, recommended dietary intake. AI, adequate intake.
[a]The AI for younger adults (19-50 years) is based on the amount of vitamin D required to maintain serum 25(OH)D at a level of at least 27.5 nmol/L with minimal exposure to sunlight.
Source: National Health and Medical Research Council and New Zealand Ministry of Health, 2006, Nutrient Reference Values for Australia and New Zealand, (http://www.nrv.gov.au/nutrients/calcium), last accessed 12/11/2015.

1.4.1.5 Prevalence of inadequate calcium intake and vitamin D deficiency in younger women

Inadequate dietary calcium intake is very common in Australia despite its abundant sources from milk and milk-based foods. In the Australian Health Survey in 2011-2012[(81)], over two in three women aged 19-50 years had inadequate calcium daily intake (based on EAR, **Table 1.3**).

Table 1.3: Proportion of population with inadequate calcium intakes in women aged 19-70 years (estimated as % below the EAR)

Age (years)	EAR (mg)[a]	Prevalence of inadequacy (%)[b]
19-30	840	71.3
31-50	840	67.2
51-70	1100	91.2

Source: (a) National Health and Medical Research Council and New Zealand Ministry of Health, 2006, *Nutrient Reference Values for Australia and New Zealand*, (http://www.nrv.gov.au/nutrients/calcium), last accessed 12/11/2015.
(b) Australian Health Survey: Usual Nutrient Intake, 2011-12.

Although severe vitamin D deficiency (defined as a serum 25(OH)D level of < 25 nmol/L), resulting in rickets and osteomalacia in children and osteomalacia in adults, is now uncommon, subclinical vitamin D deficiency (serum 25(OH)D concentrations < 50 nmol/L) has been reported worldwide. A 2014 systematic review of 195 studies from 44 countries (168 000 participants) demonstrated that 37.3% of the studies reported mean serum 25(OH)D < 50 nmol/L[82].

According to the Australian Health Survey 2011-12[83], vitamin D deficiency is highly prevalent in Australia, particularly in winter and for those living in the southern states (Figure 1.1**)**. For example, a cross-sectional study showed that almost one quarter of the population had a serum 25(OH)D level below 50 nmol/L among 2413 adults aged 24-95 years between 2008 and 2010 in South Australia[84]. A high prevalence of vitamin D deficiency was also reported in younger and middle-aged Tasmanian women aged 20 to 60 years, with more than two in three having a serum 25(OH)D level ≤ 50 nmol/L[85]. Importantly, the National Health Survey component of the Australian Health Survey showed that only 5% Australian adults were taking Vitamin D supplements in 2011-12[83].

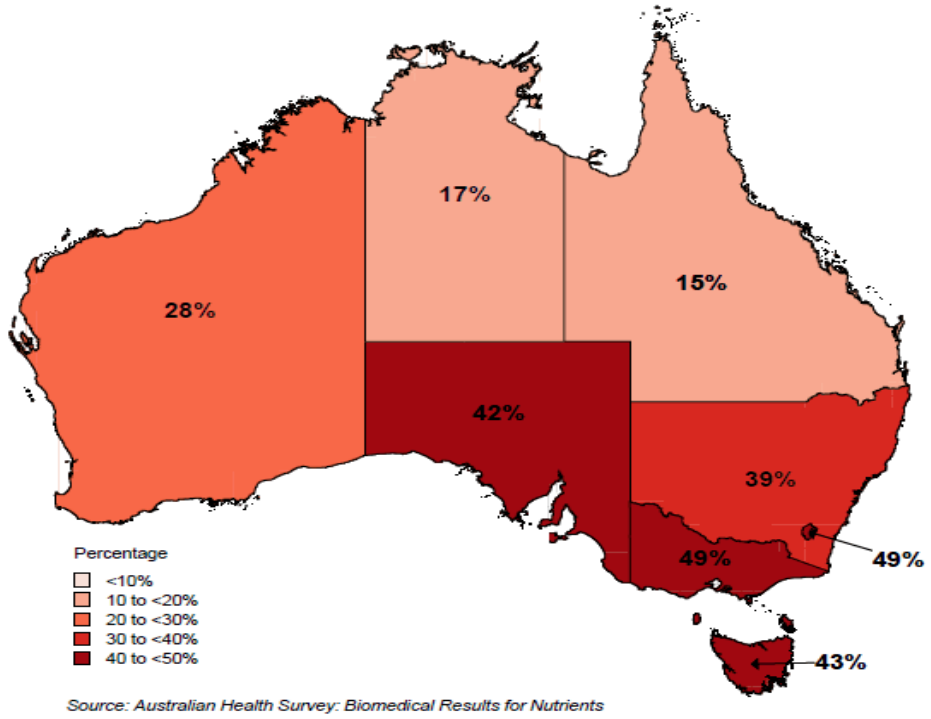

Figure 1.1: Serum vitamin D in winter in Australia by state and territory, 2011-12; Percentages indicate the proportion of vitamin D deficient populations (25(OH)D levels < 50 nmol/L) (adopted from Australian Bureau of Statistics, Australian Health Survey: Biomedical Results for Nutrients).

1.4.2 Physical activity

1.4.2.1 Physical activity and bone health in younger women

Physical activity is beneficially associated with fractures in both older[86] and younger adults[87-91]; however evidence of similar associations between physical activity and fracture outcomes in premenopausal women is lacking, due to insufficient data.

A meta-analysis of nine controlled trials showed that exercise programmes that combine odd- or high-impact activity with high magnitude resistance training was effective at lessening reduction in BMD at femoral neck and lumbar spine in premenopausal women (weighted mean difference = 0.007 g/cm^2 (95%CI: 0.001-0.013) and 0.009 g/cm^2 (95%CI: 0.002-0.015), respectively)[87]; however, the high-impact only protocols were only effective at improving femoral neck BMD (weighted mean difference = 0.024 g/cm^2 (0.002-0.027)). This suggest different types of exercise may have different effect size on various bone sites and a combined exercise program might be required to achieve the optimal benefits. Nevertheless, given that low BMD increases the relative risk of osteoporotic fracture in younger[92] and middle-aged women[93], it is reasonable to conclude that sufficient physical activity in younger women may prevent fractures.

1.4.2.2 Prevalence of insufficient activity and physical inactivity in younger women

For adults aged 18-64 years, the latest Australia's Physical Activity and Sedentary Behaviour Guidelines recommend 150-300 minutes of moderate or 75-150 minutes of vigorous physical activity, or an equivalent combination of both, per week[94]. In 2014-15, 47% to 58% of 18-64

years old women were insufficiently active (less than 150 minutes in the last week) or inactive (no exercise in the last week)[95].

1.4.3 Smoking

1.4.3.1 Health impacts of smoking on bone health in younger women

Smoking cigarettes has been associated with increased risk of fractures in older women[96]. However, results are inconclusive with regard to BMD in premenopausal women[97-101], with a meta-analysis of cross-sectional studies failing to observe differences in BMD between premenopausal smokers and nonsmokers[99]. However, this might be due to poor reporting of studies, as between-studies heterogeneity was not reported, nor were subgroup analyses were performed to assess potential effect modifiers. Indeed, the relationship between smoking and BMD could be significantly modified by BMI, breastfeeding and sports participation in premenopausal women[97]. In comparison, studies which took into account potential effect modifiers more comprehensively, showed negative associations between smoking cigarettes and BMD in younger women[97,98,100]. For example, a cross-sectional study found that current smoking was associated with significantly reduced BMD in premenopausal women (mean age 33 years), particularly those with a BMI < 25 kg/m^2 [97]. Also, the deleterious effect of breastfeeding and the beneficial effect of participating in competitive sports on BMD were only present in smokers. Therefore, it is important to consider these possible confounders in evaluating results of past studies and future research. Taken together, the evidence suggests that smoking may have a deleterious effect on BMD in premenopausal women, though this effect may exist only in certain subgroups.

Despite smoking cigarettes being considered a major risk factor for osteoporosis, evidence is limited about the mechanisms by which it exerts effects on bone[99,102,103]. Several potential

mechanisms have been proposed, including having lower body fat, earlier age of menopause, increased adrenal cortical hormones, lower serum 25(OH)D levels and calcium absorption in smokers compared to non-smokers[101]. However, evidence supporting these hypotheses is of poor quality and more work is needed to provide a clear and systematic understanding about this.

1.5 Osteoporosis knowledge and self-efficacy

1.5.1 Status of osteoporosis knowledge and self-efficacy in younger women

Numerous studies have reported that younger women had low levels of knowledge about osteoporosis[104-114], though the majority of them have been in a convenience sample of participants. Only two studies demonstrated high levels of osteoporosis knowledge[112,115]. Chang et al. showed that community-dwelling women aged 25-45 years (n = 265) answered more than 80% questions related to osteoporosis correctly[112]. Although this was a population-based study, the response rate was extremely low (16.7%), conferring a high risk of selection bias and lowering the generalizability of the findings. A recent cross-sectional study among 430 women aged 20-35 years showed similarly high levels of knowledge with women scoring a mean of 18.5 from a maximum score of 23, but the findings might be compensated by the highly-selected participants, who attended the Gynecology Clinic[115]. Overall, younger women are likely to have low levels of osteoporosis knowledge, though population-based studies are lacking.

Self-efficacy refers to “beliefs in one's capabilities to organize and execute the courses of action required to manage prospective situations”[116]. Few studies have assessed osteoporosis self-efficacy in younger women. Those that have, have used a variety of scoring scales, and reported low to moderate levels of osteoporosis self-efficacy (53%-78% of the maximum

possible scores for exercise self-efficacy, 66%-78% for calcium self-efficacy, and 41%-71% for overall self-efficacy, respectively)[109,114,117-121]. Most studies have been conducted in convenience samples of highly selected populations, with only two reported in randomly selected samples of women[114,119]. Wallace and colleagues demonstrated moderate levels of both exercise and calcium self-efficacy (63% and 68%, respectively) in a random sample of female undergraduates college students in the United States (n = 273, age range 17-64 years)[119]. Another larger study (n = 470) reported a similar but slightly higher overall osteoporosis self-efficacy (71%) in a random population-based sample of women aged 25-44 years in south Tasmania, Australia[114].

1.5.2 Relationships between osteoporosis knowledge, self-efficacy and behaviours in adult women

Both osteoporosis knowledge and self-efficacy are two key factors involved in behaviour change for osteoporosis prevention, though this is not definitely confirmed. Evidence from cross-sectional data has been conflicting in whether increased levels of osteoporosis knowledge are associated with increased participation in osteoporosis preventive behaviours[112,119,122-125]. The discrepancy might be explained by the study populations' characteristics such as age and gender. Nevertheless, these findings do not allow for drawing any conclusions about causal relationships. Though this could be ascertained in intervention studies, the findings are similarly inconclusive[120,126-135]. Although an increase in osteoporosis knowledge could be usually observed following an educational program, only a few studies found a concurrent increase in the participation in osteoporosis preventive behaviours[120,126,132,133,135]. Of note, more than half of those intervention studies had a one-group pre-post-test design, which has a number of internal validity issues (e.g., maturation

and testing). Moreover, the majority of those studies had only a short education session (less than one hour), which precludes the inclusions of a broader spectrum of topics or greater interactions between the participants and the instructor (knowledge instruction and feedback). In comparison, our previous studies found that the group-based education (the Osteoporosis Prevention and Self-Management Course, a weekly two-hours session for four weeks) could improve osteoporosis knowledge over two years and behaviours over both two and 12 years (unpublished data), compared to a simple leaflet of osteoporosis information[114,126,136]. Therefore, adequate osteoporosis knowledge could be critical for improving osteoporosis preventive behaviours but more well-designed RCTs of longer educational programs are urgently needed.

Osteoporosis self-efficacy has been related to behaviours in three ways: the conviction that an individual has the ability to i) initiate the activity, ii) maintain the activity and iii) persist in performing the activity in the face of obstacles[137]. Self-efficacy has been studied in the context of numerous diseases and behaviours, and it has been shown to be a critical contributor to improving osteoporosis preventive behaviours[119,127,135,138]. For instance, in a study aiming to test a model of certain factors influencing people engaging in osteoporosis preventive behaviours, including exercise and calcium intake, self-efficacy was a better predictor than were the other variables (i.e., age, years of education, knowledge of osteoporosis, social support and social capital)[138].

Therefore, current evidence suggests that interventions aiming at improving osteoporosis knowledge and self-efficacy may be important for promoting behaviour change for osteoporosis prevention. However, there is limited literature identifying effective innovative approaches to do this, particularly those with long-term follow-up.

1.6 Osteoporosis education and fracture risk feedback for preventing osteoporosis in younger women

1.6.1 Osteoporosis education

1.6.1.1 Public health messages and recommendations for osteoporosis prevention in Australia

In Australia, a range of clinical guidelines[139,140]recommendations[141,142] and position statements[142-144] for osteoporosis prevention have become available since 2000, the beginning of our original study[126]. At that time, adequate calcium and vitamin D intake, regular weight-bearing exercise, and cessation of cigarette smoking had been proposed for the prevention of postmenopausal osteoporosis[142,145]. In 2002, calcium intake, vitamin D, engaging in regular exercise were again emphasised as treatments of postmenopausal osteoporosis in the Australian Fracture Prevention Summit[146]. From 2005 to 2013, three position statements for vitamin D[79,144,147] and one for calcium[143] had specifically discussed their importance for bone health. In 2012, the latest recommendations for physical activity for adults had also been released[148]. In 2013, as the result of the Osteoporosis Australia Summit in 2011, building healthy bones throughout lifetime was propounded as an approach to osteoporosis prevention[149]; three conventional and affordable strategies were reconfirmed to be important for bone health throughout the lifecourse: adequate daily dietary calcium intakes, vitamin D levels, and appropriate physical activity. In 2014, the Osteoporosis Australia released a medical guide of "what you need to know about osteoporosis" to provide a comprehensive description about osteoporosis and fractures from the following aspects: epidemiology, risk factors, diagnosis, management and treatment, and the prevention of re-fracture and falls[141]. Again, adequate calcium intakes, vitamin D levels, and appropriate

physical activity were highlighted for optimising bone mass, preserving BMD and slowing bone loss.

1.6.1.2 Effects of osteoporosis education on osteoporosis knowledge and self-efficacy

Despite the important role of osteoporosis knowledge and self-efficacy in change in osteoporosis preventive behaviours, the available evidence suggests that both osteoporosis knowledge and self-efficacy are inadequate in young women (as previously described in Chapter 1.5). However, the effective interventions to change this have yet to be ascertained, certainly at a population level.

Previous studies have employed a variety of educational interventions to improve osteoporosis knowledge and self-efficacy in young women[114,120-122,131,132,135,150-153]. Most of those studies have used the form of providing brief written educational materials to participants via mail or internet (an information packet or leaflet) or a few educational sessions, and all of which demonstrated an increase in osteoporosis knowledge but only some showed an increase in osteoporosis self-efficacy[121,135,150,151]. Of note, these studies have been short-term in nature (≤ 6 months) and the majority of them have been in a convenience sample of participants, thereby limiting the generalizability of their results.

Only one RCT, the original study of the 10-yr additional follow-up study from which the data are utilised for the present thesis, has examined the effectiveness of group-based education for improving osteoporosis knowledge and self-efficacy in a population-based sample of young women (n= 470, age range 25-44 years)[114]. Specifically, participants in this study were randomly assigned to one of two educational interventions: a simple osteoporosis information leaflet produced by Osteoporosis Australia “Understanding Osteoporosis”; or the

Osteoporosis Prevention and Self-management Course (OPSMC). The OPSMC is a chronic disease self-management course developed by the Arthritis Foundation of Victoria and utilized by Osteoporosis Australia. The aim of this small-group patient education program is to increase knowledge, improve confidence and awareness and self-management of osteoporosis prevention with an emphasis on promoting appropriate lifestyle changes. OPSMC sessions of 2 hours were held weekly for 4 weeks with a maximum of 16 participants per group. The osteoporosis information leaflet, from Osteoporosis Australia "Understanding Osteoporosis", provided a comprehensive description of osteoporosis and a discussion of the role of lifestyle factors including diet, exercise and smoking, and optimal levels of calcium intake and exercise[154]. They also received individualised BMD feedback, that is, women who had a mean T-score at spine and hip of greater than or equal to 0 were not at a higher risk of fracture in later life, whereas those who had a mean T-score of less than 0 were at higher risk. After six months of the interventions, women in the OSPMC group had higher osteoporosis knowledge compared to the leaflet group and this persisted at 2 years (β = 1.33, 95% CI: 0.72-1.94 and 0.64, 95% CI: 0.0034-1.25, respectively) though the magnitude was reduced. In contrast, women who received feedback of higher fracture risk had a significant increase in osteoporosis knowledge at 2 years (β = 0.66, 95% CI: 0.0034-1.25) but not six months, compared to those who were informed of not being at a higher risk. However, osteoporosis self-efficacy was not associated with either intervention. Nevertheless, the effects of these interventions in the long term (e.g., > 5 years) remains unknown.

1.6.1.3 Effects of osteoporosis education on preventive behaviours

A number of controlled trials of a variety forms of educational interventions have been conducted in premenopausal women aimed at improving a number of osteoporosis preventive behaviours (Table 1.4)[120,126,132,151,155-163]. This is typically by targeting aspects in the causal pathway (knowledge, attitudes, and self-efficacy). Some have demonstrated improvements in exercise[160], calcium intake[156,157,159,163], intake of dairy foods[155], calcium supplements[160,163] and vitamin D intake and supplements[156,159,160] but the others did not show any benefits[120,126,132,161,162]. Overall, these studies have been short term in nature (less than or equal to 1 year) and were in a convenience sample of participants with only one exception that was our previous 2-year RCT in a randomly selected population-based sample of premenopausal women (age range 25-44 years) as described previously in Chapter 1.6.1.1[126]. In this study, women received one of two educational interventions: a simple osteoporosis information leaflet produced by Osteoporosis Australia "Understanding Osteoporosis"; or the OPSMC. At the end of the 2-year follow-up, women in the OPSMC group did not differ in the improvements in dietary calcium intake, self-reported use of calcium supplements, smoking cessation, increased physical activity, or BMD at either the femoral neck or lumbar spine, compared to the group who received the leaflet alone. Besides the educational intervention, a second intervention was provided: individualised BMD feedback i.e. women who had a mean T-score at spine and hip of greater than or equal to 0 were informed they were not at a higher risk of fracture in later life, whereas those who had a mean T-score of less than 0 were at higher risk. The outcomes of this intervention are described in detail in Chapter 1.6.2.3.

Similar to osteoporosis knowledge and self-efficacy, the long-term effects of educational interventions on osteoporosis preventive behaviours or even prevention of fractures remains unknown in premenopausal women.

Table 1.4: Characteristics of controlled trials examining effects of educational interventions for improving osteoporosis preventive behaviours in premenopausal women (listed in chronological order of publication year)

Authors (Year)	Sample size at baseline (at follow-up)	Study design	Menopausal status	Setting	Age (year)	Intervention	Duration of follow-up	Findings
Blalock et al. (2000)[132]	536 (307)	RCT	Premenopausal	North Carolina driver's license records	35-43	Group 1 (intervention): an information packet containing general information about osteoporosis; Group 2 (intervention): an action plan packet containing instructions on how to increase one's level of exercise and calcium intake; Group 3 (intervention): both packets; Group 4 (control): neither packet.	One year	No effects on calcium intake or physical activity behaviours.
Peterson et al. (2000)[163]	122 (80)	CCT	Premenopausal	Convenience sample, a university setting in Memphis, Tennessee.	18-30	Group 1 (intervention): Individualized DXA Feedback (no description how the DXA results were presented), three small group dietary education sessions, and provided with calcium supplements; Group 2 (control): usual care.	Six months	Greater increases in total calcium intake and supplemental calcium. Women in the treatment group did not experience significant changes in total BMC, but women in the control group experienced significant losses in total BMC
Ribeiro and Blakeley (2001)[162]	138 (93)	Quasi-experimental	Mixed (n = 50, premenopausal)	Community; Canadian Women's Institute.	45-69	Group 1 (intervention): Attended multidisciplinary day long education on osteoporosis with exercise practice sessions; Group 2 (control): attended workshop not related to osteoporosis.	Six months	Smoking, exercise, vitamin D supplementation, calcium supplementation did not differ at the end of follow-up. Though calcium intake was higher in intervention group, this might be due to its higher baseline level.
Brecher et al. (2002)[120]	97 (86)	RCT	Mixed (n = 23, premenopausal)	Convenience sample, southern New Jersey area through newspaper advertisements, posted notices, mailings, and word of mouth.	25-75 (66% < 60)	Group 1 (intervention): One, 3-h small group session with lecture and interactive exercises; Group 2 (control): offered educational session after the end of the study.	Three months	More participants had self-reported increased calcium intake, but no objective differences in calcium intake as actually measured.
Blalock et al. (2002)[161]	714 (547)	CCT	Premenopausal	Convenience sample, 12 counties in western North Carolina.	40-56	Intervention 1: Group 1 (intervention): Education tailored on stage of change and current behaviours; 2 mailings and 1 phone call; Group 2 (control): Mailed, 2 packets of standardized information. Intervention 2:	One year	In women with inadequate calcium intake at baseline, tailored materials increased calcium in the short term (six months) but not in the longer term (1 year), and no effects on exercise participation. Community intervention had no

						Group 1 (intervention): Community partners and stage of change provided free bone density Assessment; Group 2 (control): Community partners did not provide free BMD assessment.		effects on changes in calcium or exercise behaviours.
Kulp et al. (2004)[160]	195 (195)	RCT	Mixed	Attending a gynaecological examination in an outpatient setting. United States.	35-80	Group 1 (intervention): Educational video 10 min in length focusing on osteoporosis and prevention preceded visit with physician who was blinded to group assignment; Group 2 (control): usual care.	Three months	Increased calcium supplements, vitamin D supplements, weight-bearing exercise, and hormone therapy.
Chan et al. (2005)[159]	56 (41)	RCT	Mixed	A private beauty clinic in Hong Kong	> 18 (78% participants < 46)	Group 1 (intervention): a structured, individualized educational session and a supportive telephone follow-up programme, which covered the four behaviours (consumption of soya foods, milk and vitamin D/more exposure to sunlight and increased exercise) were discussed in one 45-minute education session, followed by two telephone consultations that were conducted within one month. Group 2 (control): no education session.	One month	Increased consumption of calcium including soya-based foods, milk and vitamin D.
Winzenberg et al. (2006)[126]	470 (415)	RCT	Premenopausal	Randomly selected in Southern Tasmania using the Tasmanian Electoral Roll as the sampling frame.	25-44	Intervention 1: Group 1 (intervention): the Osteoporosis Prevention and Self-management Course (OPSMC); Group 2 (control): an information leaflet produced by Osteoporosis Australia "Understanding Osteoporosis". Intervention 2: Group 1 (intervention): higher risk of fracture in later life (mean spine and hip T-score < 0); Group 2 (control): normal risk of fracture in later life (mean spine and hip T-score ≥ 0).	Two years	No effects on dietary calcium intake, self-reported use of calcium supplements, cessation of smoking, increased physical activity, or BMD of femoral neck and lumbar spine.
Huang et al. (2011)[158]	68 (68)	CCT	Mixed	Using posters and flyers in two community development centers in Changzhi Township, Pingtung County, Taiwan.	> 40	Group 1 (intervention): three primary components associated with the HBM, including “individual perceptions, modifying factors, and likelihood of action”. Group 2 (control): no intervention.	12 weeks	Increased intake of calcium-rich foods, weight-bearing exercise, BMD T-score after intervention, adjusted for baseline values.
Jung et al. (2011)[157]	133 (98)	RCT	Premenopausal	McMaster University in Hamilton, Ontario, Canada	18-19	Group 1 (intervention): gain-framed, targeted materials; Group 2 (control): standard osteoporosis educational materials.	One year	Increased calcium intake

Lv and Brown (2011)[156]	141 (131)	Quasi-randomized trial	Unknown	Convenience sample, first-generation Chinese-American from six weekend Chinese schools in the Philadelphia, Pennsylvania.	35-55	Group 1 (intervention): six culturally focused weekly interactive small group lesions including food preparation demonstration, personal feedback, and involvement of family; Group 2 (control): six weekly nonrelated financial lessons by mail.	Three months	Increased calcium and vitamin D intake.
Poddar et al. (2012)[155]	211 (179), 63% women	RCT	Premenopausal	College students in a university campus, Johns Hopkins.	20.2	Via electronic mail an 8-week educational intervention of: Group 1 (intervention): dairy intake; Group 2 (control): stress management.	Eight weeks	Higher intake of total dairy foods.
Jeihooni et al. (2015)[151]	120 (120)	quasi-experimental	Unknown	Patients registered in two healthcare centres (Fasa, Iran).	30-50	Group 1 (intervention): eight educational sessions of 55-60 minutes. Each session included a combination of lectures, group discussion, questions, and answers, showing posters, short videos, and PowerPoint displays. Moreover, educational pamphlets were given to the participants at the end of the last session. Group 2 (control): no intervention.	Six months	BMD T-score of lumbar spine and hip increased in the intervention group while decreased in the control group, though the difference was not statistically significant. Nutrition and jogging performance were higher in the intervention group than the control group.

RCT, randomized controlled trial; CCT, clinical controlled trial.

1.6.2 The feedback of fracture risk as a potential strategy

The feedback of fracture risk or BMD has been reported to be a potentially effective intervention to improving osteoporotic preventive behaviours, thus improving BMD[126].

1.6.2.1 BMD

The mineral content of bone can be assessed using measures of BMD. BMD can be measured by a number of approaches including quantitative computed tomography (QCT, measuring volumetric BMD in g/cm^3), single photon absorptiometry (SPA), dual photon absorptiometry (DPA), digital X-ray radiogrammetry (DXR) and single- and dual-energy X-ray absorptiometry (SXA or DXA). DXA is the international standard for the clinical assessment of bone density, and assesses areal bone mineral density in g/cm^2. Currently there is no other skeletal health assessment technology that provides as much clinical information as DXA for diagnosing osteoporosis, assessing fracture risk, and monitoring changes in BMD over time[164].

Areal BMD without clinical interpretation is unlikely to be useful in communicating with patients and/or consumers. However, bone density results can be used to classify people into diagnostic categories of osteoporosis according to T-score. T-score is the expression of individual BMD in relation to the young healthy population in standard deviation (SD) units. WHO has proposed four general diagnostic categories for osteoporosis for women based on measurements by DXA[165]. Normal: a value of BMD within one standard deviation of the young adult reference mean (T-score $\geq$ -1); Low bone mass (osteopenia): a value of BMD more than one standard deviation below the young adult mean, but less than two standard deviations below this value (T-score < -1 and > -2.5); Osteoporosis: a value of BMD 2.5

standard deviations or more below the young adult mean (T-score < -2.5); Severe osteoporosis (established osteoporosis): a value of BMD 2.5 standard deviations or more below the young adult mean in the presence of one or more fragility fractures.

1.6.2.2 The importance of BMD

BMD is a major predictor of fracture risk[16,166]. Low bone mass prior to menopause is as important as postmenopausal rate of bone loss for the risk of fracture[167]. It is therefore possible to translate bone density results into relative or absolute fracture risk assessments. For example, a prospective study showed that in postmenopausal women (n = 2161) a one standard deviation decrease in femoral BMD was independently associated with a 2.4-fold increase in relative fracture risk[166]. In contrast, absolute fracture risk can be predicted by a variety of fracture risk calculators with or without BMD[168], for example, the Fracture Risk Assessment (FRAX) tool developed by WHO. One recent prospective study in US women aged 65 or older showed that the FRAX could well predict 10-year absolute hip fracture risk both with or without BMD [the area under the receiver operating characteristics curves (AUC) values ranged from 0.62 to 0.79][169].

Thus, it is possible to provide interpretations of bone density measures to people that are likely to be meaningful and communicate potential health consequences.

1.6.2.3 How the strategy might work

Healthy risk awareness plays a key role in motivating osteoporosis preventive behaviours in preventing fracture[170]. However, individuals' awareness of[171] osteoporosis is low[172], as described earlier. Young adult women (mean age (SD) = 42.5 (0.6) years) reported that osteoporosis was not serious and it was not necessary to take preventive measures[112].

Similarly, a recent study in Australia has shown that osteoporosis as a medical condition was considered of having low salience by general medical practitioners (GPs), particularly in comparison with other diseases[171]. These highlight the importance and potential benefit of improving risk awareness of osteoporosis to both general populations and GPs.

Interventions aimed at successfully communicating disease risk are beneficial across a range of clinical areas; those including individualised risk estimation are typically more effective than other forms of personal communication[173]. Feedback of personalised information to individuals about BMD or fracture risk based is an under-explored potential osteoporosis intervention[174], which may promote osteoporotic preventive behaviours by improving individuals' health risk awareness, and thereby motivating them to improve knowledge about how to reduce risk, changing attitudes and improving self-efficacy. In addition, fracture risk and bone density feedback delivered as above can be considered a form of biofeedback.

Successful employment of biofeedback can be beneficial for health-related behaviours. A study of 261 women compared self-reported changes in behaviours of osteoporotic fracture prevention between women who were informed that their bone density results were below normal and those who were informed that their results were normal[175]. Participants who received BMD results stating that their BMD was lower than normal were much more likely to begin osteoporotic fracture preventive measures (94% vs. 56%, $p< 0.01$), to start hormone therapy (38% vs. 8%, $p< 0.01$), and to take precautions to avoid falling (50% vs. 9%, $p< 0.01$), compared to those who were labelled as having normal bone density. Several osteoporosis preventive behaviours have been demonstrated to have positive effects on BMD in younger women, such as smoking cessation[176,177], increased physical activity[178-181], calcium[126,182] and vitamin D supplementation[60,183]. Another study of 470 premenopausal

women found that those who received the feedback of high fracture risk had higher rates of increased calcium supplements use (15% vs. 3%, p<0.001) and increased physical activity (40% vs. 26%, p=0.001) as well as having a positive effect on bone density at the femoral neck (annual percentage change of 1.6 vs. 0.7, p<0.001)[126]. In addition, women who started calcium supplements (β of annual percentage change = 1.3, 95%CI: 0.49-2.17) and reported change in physical activity levels (β of annual percentage change = 0.7, 95%CI: 0.22-1.22) had significantly higher annual gain in femoral neck BMD. Thus it seems likely that BMD can be improved by the feedback of fracture risk mediated through osteoporosis preventive behaviours.

1.6.2.4 The effects of the feedback of fracture risk or BMD for improving osteoporosis preventive behaviours in younger women

There are only a few clinical trials investigating the effectiveness of fracture risk or BMD feedback in improving osteoporosis preventive behaviours in younger women, as summarised in Table 1.5. In a controlled trial, the effects of osteoporosis education and BMD testing on osteoporosis preventive behaviours were assessed in women aged 18-35 years[184]. Participants with low BMD (either lumbar spine or femoral neck Z-score ≤ -1) were informed that their bone mass was low. One year later, low BMD feedback was associated with increased calcium and vitamin D supplements but not physical activity. Another 1-yr controlled trial evaluated the effect of BMD feedback on lifestyle behaviours[154]. Women were informed that their BMD was low if they had a T-score < -1.0 at either the femoral neck or lumbar spine, otherwise they were told that their BMD was normal. At follow-up, women receiving low BMD feedback had higher rates of increased calcium supplements use and increased physical activity but not ceased smoking compared to those who received normal

BMD results. Unfortunately, these two studies did not examine the effects on BMD. A clinical controlled trial examined the effects of BMD feedback [low fracture risk (mean spine and hip T-score≥0) and high fracture risk (mean spine and hip T-score<0)] and educational interventions on osteoporosis preventive behaviours and BMD in premenopausal women[126]. At 2-yr follow-up, participants who received high fracture risk feedback were more likely than those receiving low fracture risk feedback to commence taking calcium supplements and to report changes in physical activity but not ceased smoking. Those women receiving feedback of high fracture risk had a greater increase in femoral neck BMD but not in lumbar spine BMD than those who received low fracture risk. Overall, while the evidence suggests in premenopausal women BMD or fracture risk feedback may be beneficial for osteoporosis preventive behaviours and BMD, the data is insufficient to be conclusive and, importantly, the long-term effects remain unknown.

Table 1.5: Characteristics of controlled trials examining effects of feedback of fracture risk or BMD for preventing osteoporosis in pre/peri-menopausal women (listed in order of number of study participants, highest to lowest)

Authors (Year)	Sample size at baseline (at follow-up)	Study design	Menopausal status	Age (year)	Intervention	Duration of follow-up	Outcomes
Barr et al. (2010)[185]	4800 (2375)	RCT	Unknown	45-54	BMD screening with results and appropriate advice (according to risk of osteoporosis) sending to GPs, who communicated with participants; No intervention for controls.	9 years	Self-reported use of vitamin D and calcium at the end of follow-up; fractures at wrist, vertebral, non-vertebral, and total hip.
Jamal et al. (1999)[184]	669 (669)	CCT	Premenopausal	18-35	BMD results were sent to participants with statement that bone mass was maintained, bone mass was consistent with osteopenia, or bone mass was consistent with osteoporosis. Women with low BMD received a hand-written note informing them that their bone mass was low and reinforcing them their bone mass was low.	1 year	Self-reported calcium and vitamin D supplements use at the end of follow-up; Fractures.
Winzenberg et al. (2006)[126]	470 (415)	CCT	Premenopausal	25-44	Mean spine and hip T-score: ≥ 0 normal risk of fracture in later life; <0 higher risk of fracture in later life.	2 years	Self-reported use of calcium supplements, cessation of smoking, increased physical activity, BMD of femoral neck and lumbar spine.
Jones et al. (1999)[154]	271 (256)	CCT	Premenopausal	33.5	T-score at either the femoral neck or lumbar spine: <-1.0 BMD was low, recommending that they consult their family practitioner; ≥ -1.0 normal.	≈1.3 year	Self-reported change in smoking status, use of calcium supplement, and increased physical activity.
Rimes et al. (1999)[186]	180 (176)	CCT	Mixed	32-73	BMD results were calculated as a percentage of the mean for their age: the highest 30% of the percentage "high" BMD results; the lowest 30% of the percentage "low", of whom, women with a result below the "fracture threshold" were further informed "they may have lost some bone and hence may suffer fractures in accidents more easily than women who have higher bone densities".	3 months	Self-reported use of calcium supplements and exercise at the end of follow-up.

RCT, randomized controlled trial; CCT, clinical controlled trial.

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Chapter 2: Rationale and research questions

2.1 Rationale

The public health burden both to individuals and the community arising from osteoporosis is substantial and is increasing as the population ages, particularly in women. Although a number of effective therapies are currently available, many patients are not diagnosed until fractures occur and compliance with drug therapies is poor[1]. Preventive interventions have the potential to be a simple and cost-effective way to alleviate the rapidly growing burden.

As low bone mineral density (BMD) is a major risk factor for osteoporotic fracture[2], and bone mass tracks throughout life[3,4], premenopausal women who are in the lower BMD range are likely to continue to have lower BMD during the postmenopausal period. Consequently, low premenopausal bone density, or premenopausal bone loss could contribute to elevated fracture risk in later life. Therefore, strategies to address the long-term maintenance of peak bone mass by slowing premenopausal bone loss is critical.

Similarly, muscle strength and balance are both important aspects of musculoskeletal health. Age-related loss of muscle strength is associated with decreased balance and functional limitations in older people[5,6] and impaired balance increases risk of falls[7]. Importantly, both muscle strength[8] and balance begin attenuating around 45-55 years of age[9,10], particularly in women, leading to suggestions that prevention of functional limitations, falls and fractures in older age should begin in early midlife.

2.2 Research questions

We performed a 10-yr further follow-up of our original 2-yr randomized controlled trial of osteoporosis educational interventions in a population-based cohort of 470 premenopausal Australian women aged 25-44 years. Using this data we addressed five research questions. The first two questions relate to the long-term effects of the interventions based on longitudinal data. Questions three to five are addressed using cross-sectional analyses of the 10-year follow-up data. The research questions are:

1. Is individualised BMD feedback and group education effective for improving osteoporosis knowledge and self-efficacy over 12 years?
2. Does individualised BMD feedback and group education continue to be effective for improving osteoporosis preventive behaviours as well as BMD for a further 10 years subsequent to the 2 year trial?
3. Do cut-points exist for associations between serum 25-hydroxyvitamin D (25(OH)D) and musculoskeletal health outcomes, below which greater 25(OH)D levels are associated with musculoskeletal health benefits and above which no such associations exist?
4.
    a. Are objectively-measured total physical activity, time spent at different intensities of physical activity and sedentary time associated with BMD, lower limb muscle strength (LMS) and balance measures in middle-aged women?
    b. If present, are any associations with moderate-to-vigorous physical activity (MVPA) independent of sedentary time, and vice versa?

5. Is LMS associated with balance measures in middle-aged women and are the associations non-linear, and, if so, is there evidence for thresholds where the associations change?

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Chapter 3: Methods

This chapter describes a further 10-yr follow-up of an original 2-yr randomized controlled trial. It incorporates aspects of study design, participants, and outcome measures of both the original (Section 3.1) and the present study (Section 3.2), and the methods of statistical analyses of the 10-yr follow up. Two chapters of this thesis (Chapter 4 and 5) use the longitudinal data from baseline to 12 years, while the other three (Chapter 6-8) use the cross-sectional data at 12 years.

Please note that the subsequent data chapters are presented in the form in which they were accepted by, or submitted to peer-reviewed scientific journals. Therefore, there are some differences in the way methods are presented in those papers as compared to this overview chapter due to the requirements of different journals and the details required for different analyses. The sample sizes used in individual chapters varies for each of the research questions.

3.1 Design and participants of the original trial

The detailed methods of the original study have previously been published[1,2]. Briefly, it was a two-year trial over 2000-2003, aiming to evaluate the effects of fracture risk feedback and educational interventions for improving osteoporosis knowledge, self-efficacy, preventive behaviours and bone mineral density (BMD) in premenopausal women. It included a randomised controlled trial component (random allocation to one of two educational interventions) and a non-randomised component (allocation to a fracture risk group based on bone mineral density). These interventions are described in detail below.

3.1.1 Participants

Participants were residents of Southern Tasmania, Australia. The population of the region is predominantly Caucasian, with 194,389 residents at June 1999, of whom 28,839 women were aged between 25 and 44[3].Women aged 25-44 years were randomly selected using the year 2000 Tasmania Electoral Roll as the sampling frame. The register of electors represents the most comprehensive population listing of Australian adults, as voting is compulsory in Australia. Participants were excluded if they had previous measurement of bone density, thyroid disease, renal failure, malignancy, or rheumatoid arthritis, a history of hysterectomy or were taking hormone replacement therapy, pregnant or planning pregnancy within 2 years of study entry, lactating. Ethics approval was obtained from the Royal Hobart Hospital Ethics Committee and all participants gave written informed consent. Of the 470 women recruited at baseline, 415 (88%) were retained in the study at two years.

3.1.2 Interventions

Participants were randomly assigned to receive the Osteoporosis Prevention and Self-management course (OPSMC) or an information leaflet by using a computer-generated random number list. After the randomisation of educational interventions was finished, participants also received feedback of being either at normal or high risk of fracture in later life. The classification of fracture risk is described below. Those in the leaflet information group received their feedback of fracture risk with the leaflet by mail, and those in OPSMC group received the feedback at the first session of the course. While there was no allocation concealment, allocation was implemented sequentially for each participant number with no variations to the order in which the

numbers were assigned. Randomisation was also performed prior to BMD results being known, so allocation could not be influenced by BMD status.

3.1.2.1 Fracture risk feedback

Participants had their BMD measured (Hologic QDR2000, Waltham, MA) at the spine and hip at baseline. Those with a mean spine and hip T-score of 0 or greater were informed that they had a normal risk of fracture in later life, whereas those with a mean T-score <0 were informed that they were at a higher risk. This was based on the observation that those in the lower half of the BMD distribution have threefold higher fracture risk both in later life and in the early postmenopausal period[(4)]. Although this cut-off is based on elderly women, it has been proposed that bone mass tracks throughout life[(5)], so that premenopausal women who are in the lower BMD range are likely to still have lower BMD during the postmenopausal period.

3.1.2.2 Osteoporosis education

The OPSMC is a chronic disease self-management course developed by the Arthritis Foundation of Victoria and utilized by Osteoporosis Australia. The aim of this small-group patient education program is to increase knowledge, improve confidence and awareness and self-management of osteoporosis prevention with an emphasis on promoting appropriate lifestyle changes. OPSMC sessions of 2 hours were held weekly for 4 weeks with a maximum of 16 participants per group. The osteoporosis information leaflet, from Osteoporosis Australia "Understanding Osteoporosis", provided a comprehensive description of osteoporosis and a discussion of the role of

lifestyle factors including diet, exercise and smoking, and optimal levels of calcium intake and exercise[6].

3.1.3 Measurements

A summary of when the measures used in this thesis were assessed in the original (baseline to two years) and the present study (12 years, see below Chapter 3.2.2) is shown in Table 3.1.

Table 3.1: Summary of when measures were assessed over 12 years [a]

	Baseline n = 470	1 year n = 463	2 years n = 415	12 years n = 347
Outcomes				
Bone mineral density	√		√	√
Osteoporosis knowledge [b]	√		√	√
Osteoporosis self-efficacy [b]	√		√	√
Change in behaviours				
Smoking status		√	√	√
Physical activity		√	√	√
Calcium intake		√	√	√
Calcium supplement use		√	√	√
Lower limb muscle strength	√		√	√
Balance				
Timed up and go test				√
Step test				√
Functional reach test				√
Lateral reach test				√
Other factors				
Height, weight, body mass index	√	√	√	√
Marital status	√		√	√
Employment status	√		√	√
Blood taken (vitamin D)				√
Accelerometer (seven consecutive days)				√
Calcium intake	√	√	√	√
Behaviours				
Smoking	√		√	√
Strenuous and light physical activity	√	√	√	√
Time watching TV or videos	√	√	√	√
Calcium supplement use [c]	√	√	√	√
Vitamin D supplement use [d]				√
Breastfeeding history	√			√
Sun exposure	√		√	√
Family history of osteoporosis/fracture	√			√
Fracture history in the participant	√			√

[a] Only the most relevant measures investigated in the original and the present study are included.
[b] Osteoporosis knowledge and self-efficacy were also measured at six weeks.
[c] At 12 years, participants were also asked to recall if they had regularly used calcium supplement at each year during the last 12 years.
[d] At 12 years, participants were asked to recall if they had regularly used vitamin D supplement at each year during the last 12 years.

Subsection 3.1.3.1 and 3.1.3.2 give detail on the factors measured in the original trial. Details of measures in the 12 years follow-up are given in sections 3.2.2.1 and 3.2.2.2.

3.1.3.1 Outcome factors in original trial

1. Osteoporosis knowledge was measured by the Osteoporosis Knowledge Assessment Tool (OKAT) (12). The OKAT has 20 items, each having true, false and don't know options. Scoring was 1 for a correct response and 0 for an incorrect or do not know response. The possible range of scores was 0 to 20. The questionnaire had a Ferguson's sigma of 0.96, a Cronbach's alpha of 0.70 and factor analysis consistent with only one factor (osteoporosis knowledge) being measured.
2. Osteoporosis self-efficacy was measured by the osteoporosis self-efficacy scale (OSES) (13) and has 6 items in each of two subscales, one relating to calcium intake and one relating to physical activity. We used a four point adjectival scale modification of the original scale, with ratings of: not at all confident (score 1), mildly confident (2), confident (3) and very confident (4). The range of possible scores was from 12 to 48.
3. BMD at the femoral neck and lumbar spine was measured by a Hologic QDR2000 densitometer (Waltham, MA), which was calibrated daily with coefficient of variation (CV) 1%.
4. Self-reported changes in osteoporosis preventive behaviours were measured by asking participants to indicate if they have changed their smoking, dietary calcium intake, calcium supplement use and physical activity at one and two years.

5. Calcium intake was assessed by a short food frequency questionnaire which has been validated against 4 day weighed records and correlates well up to 12 months(7).
6. Light and strenuous physical activity was assessed by a questionnaire validated in US adolescents(8) which we modified for Tasmanian conditions and had used previously in women of this age where it was associated with bone mass at the femoral neck(6).

3.1.3.2 Other study factors

1. Anthropometrics (age, height, weight, body mass index). Height was measured by stadiometer and weight measured by a single set of calibrated scales. Body mass index was calculated (weight (kg)/height $(m)^2$).
2. Smoking history (current/former/never, cigarettes per day, age at uptake, age at ceasing).
3. Breastfeeding history (ever breastfed, time since last breastfeeding).
4. Sun exposure - by questionnaire measuring weekday and weekend sun exposure in summer and in winter, each in 4 categories (< 2, 2-3, 3-4 and > 4 hours per day).
5. Lower limb muscle strength (LMS) was measured to the nearest kilogram using a dynamometer (TTM Muscular Meter, Tokyo, Japan)[(9)]. This test examines isometric strength, predominantly of the quadriceps and hip extensors. The examiner demonstrated the correct technique to the participant before testing. Participants stood on the back of the dynamometer platform, with back straight against a wall and knees flexed to an angle of 115 °. They

held a bar, connected to the dynamometer by a chain, and lifted the bar using maximum force using their legs, with the back and neck straight. Two readings were made, and the mean calculated for analysis.

6. Family history of osteoporosis and or fracture, and fracture history in the participant.
7. Socioeconomic factors: including employment status of participant and of main financial provider in the household and marital status.

3.2 Design and participants of the present study (additional 10-yr follow-up)

3.2.1 Participants

The present study was an additional 10-yr follow-up of the original 2-yr RCT described above (Chapter 3.1). All 470 participants from baseline in the original study were invited to participate, with 347 (74%) retained at 12 years. Ethics approval was obtained from the Human Research ethics Committee (Tasmania) Network (Approval number H11613: Strategies to address the long term maintenance of bone density in younger women: fracture risk feedback and vitamin D). We obtained written informed consent from all participants.

3.2.2 Measurements

The measures taken at the 12 year follow-up, as summarised in Table 3.1 are listed here. The methods for each measure are described in more detail in section 3.1.3 above where the measures were the same as taken at baseline, and in the relevant results chapters for

those measures performed only at 12 years.

3.2.2.1 Outcomes

1. Osteoporosis knowledge and self-efficacy (as described in Chapter 3.1.3).
2. BMD at femoral neck and lumbar spine (as described in Chapter 3.1.3).
3. Changes in osteoporosis preventive behaviours (see Chapter 5.2 for details of how they were defined):

    a. Calcium intake (increased/decreased) and calcium supplement use (never supplement/commenced or persistent supplement/cessation);
    b. Strenuous and light physical activity (unchanged/increased/decreased);
    c. Use of vitamin D supplements (no recent use/recent use);
    d. Smoking status (never smoked/cessation/commenced or persistent smoking).

4. Lower limb muscle strength was measured as described in Chapter 3.1.3.
5. Balance was measured using four clinical balance tests - the timed up and go test, the step test, the functional reach test and the lateral reach test (see Chapter 6.2 for details).

3.2.2.2 Other study factors

1. Serum 25-hydroxyvitamin D (25(OH)D) levels (see Chapter 6.2 for details).
2. Time spent in moderate-to-vigorous and light physical activity, and time spent sedentary over 7 consecutive days by accelerometer (ActiGraph GTlM) (see Chapter 7.2 for details).

3. Self-reported strenuous and light physical activity (as described in Chapter 3.1.3).
4. We determined fracture history by questionnaire. Data were collected on fracture site, age at fracture, circumstances surrounding fracture and an estimation of degree of trauma involved.
5. Medication history.
6. Anthropometrics, socioeconomic factors, date of last menstrual period and sun exposure (as described in Chapter 3.1.3).

3.2.3 Statistical analyses

3.2.3.1 Power calculation

Given that the present study was a follow-up of the previous RCT and *a prior* power calculation could not be done by calculating the number of participants we needed, we alternatively determined the minimal detectable difference for the most important outcomes based on existing data from the original study, that is, BMD of femoral neck and lumbar spine and behavioural outcomes (use of calcium supplement and increased physical activity). All calculations assume control and interventions groups of size 180, a Type I error rate of 5% and Type 2 error rate of 20%. We assumed that the low risk group mean (SD) or % (as appropriate) persists for each variable. The detectable differences for femoral neck and lumbar spine BMD (Table 3.2) are equivalent to very small yearly changes (0.21 and 0.10% p.a. for the femoral neck and lumbar spine respectively). The proportion of participants in the low risk group who take calcium supplements and report increased physical activity and the proportion detectable in the high risk group are given in Table 3.3. These are smaller than we

observed at 2 years (14.7% for calcium supplements and 40.3% for changes in physical activity in the high risk group).

Table 3.2: Detectable differences in BMD outcomes between fracture risk feedback groups

	Change in BMD at 2 years mean (SD) (g/cm^2)		Detectable difference in BMD change between groups (g/cm^2)
	Low risk	High risk	
Femoral neck	0.01220 (0.04478)	0.02132 (0.08050)	0.0193
Lumbar spine	-0.0002 (0.04561)	0.0009 (0.03114)	0.0113

Table 3.3: Detectable differences in behavioural outcomes between fracture risk feedback groups

Outcome	Low risk group (%)	Proportion in high risk group detectable at 12 years (%)
Take Calcium supplement	2.94	8.47
Increased Physical activity	25.4	12.96 or 38.49*

3.2.3.2 Statistical analysis

Statistical significance was determined using p-value < 0.05 and two-tailed tests throughout the thesis. Detailed descriptions of statistical analyses performed are presented in the relevant results chapters. All statistical analyses were performed on Stata 12 for Windows (StataCorp, College Station TX, USA).

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Chapter 4: Effects of individualized bone density feedback and educational interventions on osteoporosis knowledge and self-efficacy: a 12-yr prospective study

4.1 Introduction

Osteoporosis is a major public health problem worldwide. The financial burden on the health system it causes is increasing dramatically. For instance, in Australia the total health expenditure for osteoporosis and osteopenia in individuals over 50 years old was $2.75 billion in 2012 and it is predicted that this will increase to $3.84 billion in 2022[(1)]. Low bone mineral density (BMD) is a major risk factor for osteoporotic fracture[(2)]. Since BMD in later life is a function of peak bone mass and the rate of subsequent bone loss[(3)], it is therefore critical to ensure preventative behaviours are taken up in younger populations that improve and maintain BMD, and consequently delay the onset of osteoporosis and reduce the risk of fracture.

Osteoporosis knowledge and the concept of self-efficacy are two key factors involved in lifestyle behaviour change related to osteoporosis prevention. Self-efficacy refers to "people's confidence in their ability to change osteoporotic preventive behaviours, specifically calcium intake and physical activity", which is related to behaviours in three ways: the conviction that an individual has the ability to i) initiate the activity, ii) maintain the activity and iii) persist in performing the activity in the face of obstacles[(4)]. Both osteoporosis knowledge and self-efficacy are suggested to be important determinants of calcium intake and exercise behaviours[(5)] . Despite this, levels of

osteoporosis knowledge[6,7] and self-efficacy[6,8,9] are low worldwide. Studies suggest that osteoporosis knowledge and self-efficacy can be improved by a variety of interventions, at least in the short-term (up to 2 years). We previously[7] examined the effect of individualised risk feedback based on bone density and group education (the Osteoporosis Prevention and Self-management Course (OPSMC)) on osteoporosis knowledge and osteoporosis self-efficacy in premenopausal women. In that study, women with T-score < 0 who were told they were at higher risk of fracture in later life, based on data showing that those in the lower half of the bone mineral density distribution have a threefold higher fracture risk in later life[10] had a greater increase in osteoporosis knowledge at 6 weeks and 2 years compared to those who were told they were not at higher risk (T-score≥0). Similarly, receiving the OPSMC was associated with a greater increase in osteoporosis knowledge compared to receiving an osteoporosis information leaflet. However, neither T-score group nor type of education received was associated with changes in osteoporosis self-efficacy over 2-years.

For early life interventions to be effective at preventing osteoporosis in later life, their effects need to persist in the long-term, but there are no published studies, to our knowledge, assessing the very long-term effect of either risk feedback or osteoporosis education on osteoporosis knowledge and self-efficacy. Therefore, the aim of this study was to conduct a 12-year follow-up of participants from our original trial to determine whether the effect of fracture risk feedback and the OPSMC on osteoporosis knowledge persisted, and which, if any, factors affect osteoporosis self-efficacy in the longer-term.

4.2 Materials and Methods

4.2.1 Participants

This was a 12-yr follow-up of a randomized controlled trial previously conducted in 2000 in Southern Tasmania, Australia, the methods of which have already been described in detail[7]. We randomly selected women aged 25-44 years, from the 2000 electoral roll, excluding women if they had previous measurement of bone density, thyroid disease, renal failure, malignancy, or rheumatoid arthritis, a history of hysterectomy or were taking hormone replacement therapy, pregnant or planning pregnancy within 2 years of study entry, or lactating. Ethics approval was obtained from Royal Hobart Hospital Ethics Committee and all participants gave written informed consent.

4.2.2 Interventions assignment

A computer-generated random number list was used to randomly assign participants to one of two osteoporotic education groups: an information leaflet from Osteoporosis Australia "Understanding Osteoporosis" or the Osteoporosis Prevention and Self-management course (OPSMC). The OPSMC is a chronic disease self-management course developed by the Arthritis Foundation of Victoria and utilized by Osteoporosis Australia. This small-group patient education program aimed to increase knowledge, improve confidence and awareness and self-management of osteoporosis prevention with an emphasis on promoting appropriate lifestyle changes. OPSMC sessions of 2 hours were held weekly for 4 weeks with a maximum of 16 participants per group. Four trained educators from Arthritis Tasmania performed this using the same

materials. The osteoporosis information leaflet provided a comprehensive description of osteoporosis and discussed the role of lifestyle factors including diet, exercise and smoking, and optimal levels of calcium intake and exercise[11].

BMD at the spine and hip was measured (Hologic QDR2000, Waltham, MA) at baseline. Participants with a mean spine and hip T-score<0 received a letter informing them that their results indicated that they were at higher risk of fractures in the future and encouraging them to discuss the results and treatment options with their general practitioner, whereas those with a mean T-score≥0 were informed that they were not at a higher risk. The cut-off of a T-score of 0 was chosen based on data showing that those in the lower half of the BMD distribution have threefold higher fracture risk both in later life and in the early postmenopausal period[10]. Data specific to a younger population was not available but as evidence suggests that bone mass tracks throughout life as has been recorded in children[12], young[13], middle-aged and aged population[14], premenopausal women who are in the lower BMD range are likely to still have lower BMD during postmenopausal period.

Participants randomized to the leaflet information group received their feedback of fracture risk with the leaflet by mail, and those in OPSMC group received the feedback at the first session of the course.

4.2.3 Measurements

Osteoporosis knowledge was measured at baseline, 6 weeks, 2 years, and 12 years using the Osteoporosis Knowledge Assessment Tool (OKAT) which has previously been validated with demonstrated good discriminatory power (Ferguson's sigma =

0.96) and Cronbach's alpha =0.70[15]. The OKAT has 20 questions with true, false, and don't know options for each. Scoring was 1 for a correct answer or 0 otherwise. The possible range of total scores was 0 to 20.

The osteoporosis self-efficacy scale (OSES)[4] was used to measure osteoporosis self-efficacy at baseline, 1 year, 2 years, and 12 years. The OSES has two subscales with 6 items each for calcium intake and physical activity. We used a four point adjectival scale with ratings of: not at all confident (score 1), mildly confident (score 2), confident (score 3) and very confident (score 4). The possible range of total scores was 12 to 48.

Other study factors measured at baseline included height by stadiometer (The Leicester height measure, Invicta Plastics Ltd, Oadby, England) and weight by a single set of calibrated scales (Heine, Dover NH USA). Body mass index was calculated [weight (kg)/height^2 (m^2)]. Smoking history, breastfeeding history, number of children, family history of osteoporosis and/or fracture, and fracture history in the subject, education level, employment status of main financial provider in the household, and marital status were measured by questionnaire. Calcium intake and calcium supplement use were assessed by a validated short food frequency questionnaire[16]. The calcium content of food categories was determined by Australian food composition tables[17]. Participants who reported taking a supplement containing calcium alone or as a main ingredient at least 4 times per week were classified as taking calcium supplements. Physical activity was assessed by a questionnaire validated in American adolescents[18], which we modified for Tasmanian conditions and had used previously in women of this age[19]. This asked

participants how many days in the last 14 they performed at least 20 minutes of strenuous exercise and light exercise in five categories (1 = 0 days, 2 = 1-2 days, 3 = 3-5 days, 4 = 6-8 days, 5 = 9 or more days).

4.2.4 Statistical analysis

Differences in baseline characteristics between participants who did and did not complete follow-up were tested by unpaired two-sample *t*-test, the Kruskal-Wallis test, or chi-squared test as appropriate. Linear mixed-effect models were used to test: a. the effects of feedback of high fracture risk and of the OPSMC on change in knowledge and self-efficacy from baseline to 12 years; b. the within group change in knowledge and self-efficacy from baseline to 12 years for each T-score group (T-score<0 ≥0) and educational intervention (leaflet and OPSMC). Linear regression was used to determine the predictors of changes in knowledge and self-efficacy scores from baseline to 12 years by using complete cases. To handle missing data, complete cases were weighted by the inverse of their estimated probability of being observed[20]. All analyses were performed in Stata version 12 (Stata Corporation, Texas, USA). A two-tailed p value <0.05 was considered statistically significant.

4.3 Results

A total of 470 women (a 64% response rate) aged 25 to 44 years were recruited at baseline with 74% (347) included at year 12. Three women withdrew before bone density and baseline assessments were performed. Baseline characteristics of the remaining 467 women who did and did not complete the follow-up are presented in

Table 4.1. Participants who were lost to follow-up were younger, had a lower level of education, and were more likely to be current smokers or to have ever smoked and less likely to be married or in a de facto relationship (a non-married couple living together on a genuine domestic basis) than those completing follow-up. However, the proportions of participants receiving each intervention and other characteristics were comparable. The comparison of baseline characteristics of each intervention group has been previously published[7] and the 248 participants receiving the information leaflet had lower baseline levels of knowledge than the 219 who received the OPSMC in spite of randomization (8.4 for both T-score≥0 and leaflet group and T-score<0 and leaflet group, 9.4 and 9.1 for T-score≥0 and OPSMC group and T-score<0 and OPSMC group, respectively). Other characteristics were comparable between groups.

Table 4.1: Comparison of baseline characteristics, osteoporosis knowledge and self-efficacy of participants who did and did not complete the study

Characteristic	Completed study (*N*= 347) n (%)	Withdrawals (*N*= 120) n (%)	*P*-value
Age (yr.) (mean±SD)	38.3±5.2	36.3±5.6	<0.001
Mean T-score <0	177 (51)	60 (50)	0.892
Received OPSMC	160 (46)	60 (50)	0.462
Knowledge (mean±SD)	9.0±3.3	8.4±3.2	0.107
Self-efficacy (mean±SD)	34.5±7.1	33.8±7.0	0.334
Height (cm) (mean±SD)	163.5±6.3	162.1±6.6	0.084
Weight (kg) (mean±SD)	69.6±13.4	69.3±14.3	0.819
BMI (kg/m^2) (mean±SD)	26.1±4.8	26.3±4.9	0.432
Education level			0.038
≤Grade 10	104 (30)	52 (43)	
Grade 11-12	76 (22)	22 (18)	
>Grade 12	167 (48)	47 (39)	
Provider unemployed	21 (6)	8 (7)	0.719
Employment status			0.473
0 h/wk.	45 (13)	20 (17)	
≤20 h/wk.	83 (24)	25 (21)	
>20 h/wk.	219 (63)	75 (62)	
No. of children median	2	2	0.192
Family history of osteoporosis	58 (17)	22 (18)	0.685
Family history of fracture	129 (37)	49 (41)	0.477
Prevalent fracture	99 (29)	36 (30)	0.759
Current smoker	43 (12)	36 (30)	<0.001
Ever smoked	157 (45)	69 (58)	0.022
Married or *de facto*	264 (76)	78 (65)	0.026

Figure 4.1 gives the changes in osteoporosis knowledge over the 12-yr follow-up by (a) T-score group and (b) education group. Overall, knowledge at 12 years was higher than at baseline in all groups (10.5±3.3 vs. 8.8±3.4 for T-score<0 group; 10.2±3.2 vs. 8.9±3.2 for Tscore≥0 group; 10.9±3.1 vs. 9.3±3.4 for the OPSMC group; 9.8±3.3 vs. 8.4±3.2 for leaflet group; p<0.001 for all). As previously reported [7], compared to participants with T-score ≥0, participants with T-score < 0 had a significantly greater increase in knowledge at 6 weeks (4.0±3.4 vs. 3.4±3.3, p=0.03) and 2 years (2.8±3.2 vs. 2.1±3.1, p=0.02). In the present study, the between-group difference did not

persist at 12 years (change in knowledge of 1.6±3.4 vs. 1.1±3.4; β=-0.4, 95%CI=-0.3 to 1.1). There was no difference in the change between groups over time (p-value for group by time interaction=0.286). Similarly, participants who received the OPSMC had a significantly greater increase in knowledge at 6 weeks (4.4±3.4 vs. 3.1±3.2, p<0.001) and 2 years (2.7±3.4 vs. 2.2±2.8, p=0.048) but not at 12 years (1.4±3.5 vs. 1.3±3.4; β=0.2, 95% CI=-0.5 to 0.9) compared to participants receiving information leaflet. There was no difference in the change between groups over time (p-value for group by time=0.534), namely, no effects of interventions on knowledge.

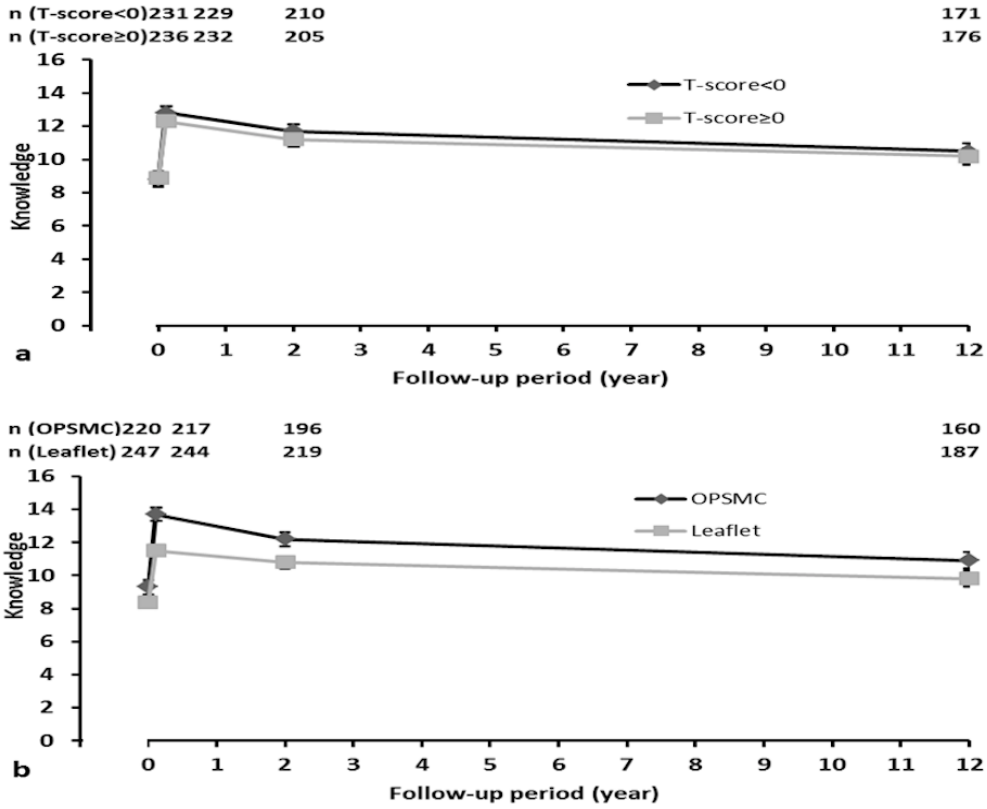

Figure 4.1: Change in osteoporosis knowledge score by (a) T-score group and (b) educational intervention (The error bars are 95% confidence interval).

Figure 4.2 shows the changes in osteoporosis self-efficacy over 12 years by T-score group and by education group. Osteoporosis self-efficacy at 12 years remained lower

than at baseline regardless of T-score group or educational intervention but the difference was small and only reached statistical significance in the low T-score group (32.9±6.9 vs. 34.0±6.9, p=0.014 for T-score<0 group; 34.5±7.8 vs. 34.7±7.3, p>0.05 for T-score≥0 group; 33.6±7.0 vs. 34.4±6.8, p>0.05 for the OPSMC group; 33.8±7.7 vs. 34.3±7.3, p>0.05 for leaflet group). The differences in change in self-efficacy between T-score groups were not significant at either 1 or 2 years as reported in previous study[7] (-1.4±7.1 vs. -1.4±5.9 at 1 year, -1.7±6.6 vs. -1.1±6.2 at 2 years, for T-score<0 and T-score≥0 groups, respectively; p>0.05 for all) or at 12 years in the current study (-1.3±6.8 vs. -0.4±6.4 for T-score<0 and T-score≥0 groups, respectively; β=-1.1, 95% CI=-2.5 to 0.4, p for group by time=0.150). Similarly, there were no significant differences in the decrease in self-efficacy between educational intervention groups at either 1 or 2 years (-1.7±6.4 vs. -1.1±6.7 at 1 year, -1.5±6.4 vs. -1.3±6.4 at 2 years for the OPSMC and leaflet groups, respectively; p>0.05 for all) or 12 years (-1.1±6.1 vs. -0.6±7.1 for the OPSMC and leaflet groups, respectively; β=-0.2, 95%CI=-1.6 to 1.3, p for group by time=0.805).

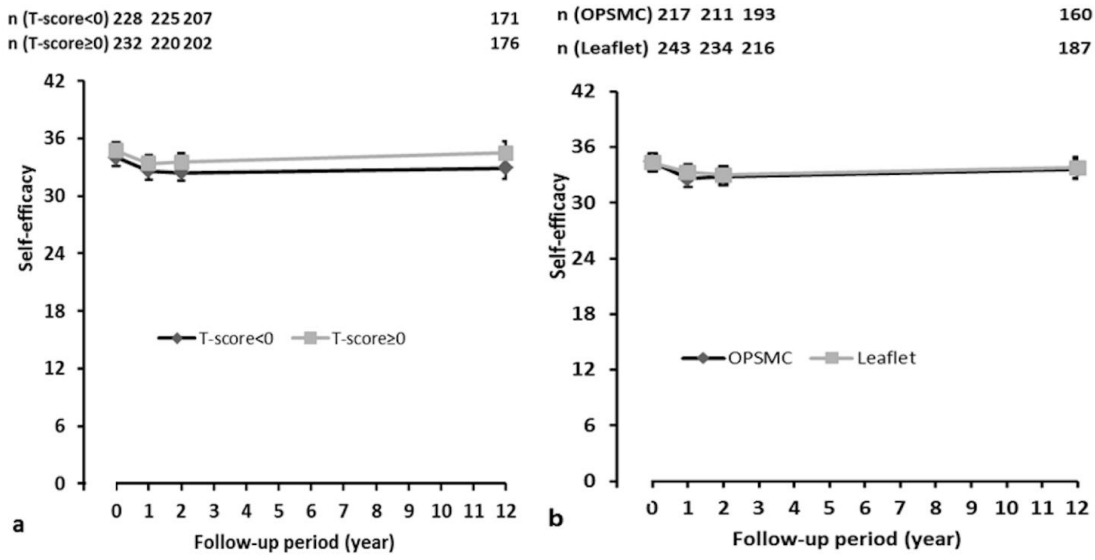

Figure 4.2: Change in osteoporosis self-efficacy score by (a) T-score group and (b) educational intervention (The error bars are 95% confidence interval).

The results of regression of potential factors affecting changes in knowledge and self-efficacy over 12 years are given in Table 4.2. Compared to baseline, women in households with an unemployed main financial provider had a slight decrease (-0.3, 95%CI=-1.7 to 1.1) in knowledge at 12-years compared to those in household with an employed main financial provider where knowledge was increased (1.4, 95% CI =1.1 to 1.8) (β=-1.95, 95%CI=-3.40 to -0.50). No other factors had a significant effect on change in knowledge over 12 years. Neither intervention nor any sociodemographic factors were associated with 12-year change in osteoporosis self-efficacy. The results did not materially change when inverse probability weighting was not used (**Supplemental Table 4-1**).

Table 4.2: Factors affecting changes in knowledge and self-efficacy over 12 years with inverse probability weighting

	Univariable β (95% CI)	Multivariable β^b (95% CI)
Knowledge		
Ever smoked	0.41 (-0.32, 1.14)	0.55 (-0.18, 1.29)
No. of children	0.10 (-0.21, 0.41)	-0.05 (-0.43, 0.33)
Employment level		
0 h/wk	Reference	Reference
≤20 h/wk	0.58 (-0.61, 1.77)	0.30 (-0.88, 1.49)
>20 h/wk	-0.07 (-1.14, 1.00)	-0.67 (-1.77, 0.43)
Provider unemployed	**-1.66 (-2.96, -0.35)** [a]	**-1.95 (-3.40, -0.50)** [a]
OPSMC	0.16 (-0.58, 0.89)	0.28 (-0.45, 1.02)
T-score group	0.47 (-0.26, 1.20)	0.42 (-0.33, 1.16)
Self-efficacy		
Ever smoked	-0.10 (-1.64, 1.43)	-0.02 (-1.56, 1.53)
No. of children	-0.30 (-0.91, 0.30)	-0.26 (-1.00, 0.48)
Employment level		
0 h/wk	Reference	Reference
≤20 h/wk	-2.56 (-5.21, 0.09)	-2.59 (-5.31, 0.13)
>20 h/wk	-0.96 (-3.21, 1.30)	-1.40 (-3.85, 1.05)
Provider unemployed	-0.68 (-5.93, 4.56)	-0.60 (-5.71, 4.50)
OPSMC	-0.28 (-1.77, 1.21)	-0.23 (-1.78, 1.32)
T-score group	-1.07 (-2.57, 0.44)	-0.90 (-2.39, 0.59)

[a] Bold denotes P<0.05.
[b] Adjusted for other items in table, education level, age, and fracture history.

Supplemental Table 4-1 Factors affecting changes in knowledge and self-efficacy over 12 years without inverse probability weighting

	Univariable β (95%	Multivariable β^b (95%
Knowledge		
Ever smoked	0.33 (-0.40, 1.05)	0.48 (-0.26, 1.22)
No. of children	0.08 (-0.21, 0.37)	-0.04 (-0.38, 0.30)
Employment level		
0 h/wk	Reference	Reference
≤20 h/wk	0.49 (-0.76, 1.74)	0.26 (-1.00, 1.53)
>20 h/wk	-0.04 (-1.16, 1.07)	-0.63 (-1.80, 0.55)
Provider	**-1.74 (-3.28, -0.20)** [a]	**-2.04 (-3.69, -0.39)** [a]
OPSMC	0.12 (-0.60, 0.85)	0.26 (-0.47, 0.99)
T-score group	0.54 (-0.18, 1.26)	0.44 (-0.28, 1.17)
Self-efficacy		
Ever smoked	-0.25 (-1.67, 1.17)	-0.15 (-1.62, 1.32)
No. of children	-0.31 (-0.88, 0.27)	-0.26 (-1.00, 0.48)
Employment level		
0 h/wk	Reference	Reference
≤20 h/wk	-2.31 (-4.73, 0.10)	-2.32 (-4.80, 0.16)
>20 h/wk	-1.04 (-3.19, 1.11)	-1.41 (-3.73, 0.91)
Provider	0.11 (-2.89, 3.12)	0.09 (-3.15, 3.32)
OPSMC	-0.45 (-1.86, 0.96)	-0.39 (-1.83, 1.05)
T-score group	-0.88 (-2.29, 0.53)	-0.79 (-2.22, 0.64)

[a]Bold denotes P<0.05.
[b]Adjusted for other items in table, education level, age, and fracture history.

4.4 Discussion

This 12-yr prospective population-based study is the first study (that we know of) to evaluate the long-term effects of any intervention for improving osteoporotic knowledge and/or self-efficacy in premenopausal women. While both the OPSMC and feedback informing women they were at higher fracture risk improved osteoporosis knowledge for 2-years post-intervention, these beneficial effects did not persist in the long-term. Nonetheless, overall knowledge increased in all intervention

groups over 12 years, suggesting that these increases were due to factors other than the interventions provided. Neither intervention improved osteoporosis self-efficacy at 2 or 12 years. These results suggest that more frequent osteoporosis education and bone density feedback may be necessary to maintain the increased short-term knowledge gains from these interventions, though there are no current data that allow an estimate of optimal frequency to be made. This requires further investigation. The results also suggest that alternative interventions are required to improve self-efficacy.

Previous studies have employed a variety of educational interventions to improve osteoporotic knowledge, but only a few have been conducted in younger women[21,22], and only two have specifically investigated the OPSMC[6,8] in addition to our previous 2-yr study[7]. Overall, these studies have been short-term in nature (≤3 months) and participants were not selected using random sampling[6,8,21,22], thereby limiting the generalizability of their results. The two studies in younger women (age≤25) reported that brief education interventions could produce moderate increases in knowledge (up to 44%) in the short-term (≤4 weeks)[6] Studies using the OPSMC in older populations (average age>60, up to 3 months follow-up[8] and people aged 40 or over (92% women) with 6 weeks follow-up[6] showed similar short-term effects of the OPSMC on knowledge. These are similar to the short-term increases we previously reported in our younger study population[7].

Osteoporosis knowledge decreased after 6 weeks across all groups but was still higher than at baseline after 2 and 12 years. While this may be due to the effects of the interventions, it is also possible that this reflects the natural history of osteoporosis knowledge acquisition with increasing age, and presumably increasing awareness of

the issue of osteoporosis. Even though women in households with an unemployed main financial provider only accounted for 6% of our study participants, they were more likely to have a slight decrease in osteoporosis knowledge compared to those with an employed main financial provider where knowledge was increased. It may be that women living in such households would place less emphasis on osteoporosis due to financial circumstances, and/or they may have limited access to educational resources, healthcare providers and other societal supports that could assist them in acquiring and maintaining knowledge of osteoporosis[23]. Future research on improving osteoporosis knowledge should focus on this population.

Only two other studies have evaluated the short-term (up to 3 months) effects of the OPSMC on self-efficacy[6,8] other than our previous 2-yr study, but these two studies are not directly comparable with ours because they had a shorter duration of follow-up (maximum of 3 months) and included older participants (mean age>63). Nonetheless, as in our previous 2-yr study[7] and in our current data, these two studies reported no significant change in self-efficacy from the OPSMC (4% for both studies) as compared to the no intervention (1%)[6] or one session course control group (3≤%)[8]. Thus it seems that the OPSMC may not be an effective approach for changing osteoporosis self-efficacy in either younger or older women. Information on the effect of fracture risk feedback on osteoporosis knowledge is limited. One study reported no significantly higher increase in osteoporosis knowledge after 4 months in peri- or post-menopausal women (mean age=54) who received immediate verbal information from the consultant regarding BMD results, diagnosis and implications compared to those who received a standardized letter regarding the results only from

their general practitioner (13% vs. 28%)[24]. This may be due to no educational intervention being provided.

As we observed in our study at 2 years follow-up[7], changes in osteoporosis self-efficacy over12-year were independent of whether participants had received feedback of high fracture risk. We also observed that the negative associations of both 1-year and 2-year changes in osteoporosis self-efficacy with number of children and hours of employment were no longer significant. This short-lived relationship might be a result of the lengthy study period as employment status might have changed and the impact of the number of children on daily routines is likely to change as children grow up and become independent.

Our study has several important strengths. The educational intervention component of the study was a robustly designed randomized controlled trial. In addition, our study was population-based with randomized sampling to ensure a low selection bias and so a high generalizability. The large sample size allowed us to have a very long-term follow-up with sufficient statistical power for these analyses. Furthermore, the 12 years follow-up made our study unique as no other relevant studies have been conducted for such a long period.

A limitation of the study is that follow–up was incomplete, with 26% of participants lost to follow-up over 12 years. However, the proportions of participants receiving the OPSMC and low T-score feedback were comparable between those who did and did not complete the study suggesting that the risk of bias from loss to follow up is low and unlikely to have affected study findings. This is supported by data from analyses using linear mixed model and inverse probability weighting to adjust for

missing data, as the results did not vary from those without using inverse probability weighting.

The OPSMC was modelled upon a chronic disease self-management course for arthritis effective in symptomatic populations[25,26], which are likely to be different from the healthy, asymptomatic participants in the current study. However, a recent study indicated that the OPSMC intervention led to a significant increase in osteoporosis knowledge but not osteoporosis self-efficacy for calcium or exercise in adults aged ≥ 50 having sustained an acute bone fracture due to minimal trauma[8]. This suggests the OPSMC is effective for improving knowledge but not self-efficacy in both symptomatic and healthy populations. A more specific education session focusing on improving self-efficacy may be useful, and it should be designed based on the four main sources that form and affect self-efficacy: personal accomplishment, verbal persuasion, vicarious experience, and physiological or affective states[8].

In conclusion, in this population-based randomized controlled trial in healthy women aged 25-44, both the OPSMC and high fracture risk feedback increased osteoporosis knowledge over 2 but not 12 years. Women in households with unemployed main financial providers were more likely to have decreased knowledge in a long-term, suggesting that this population should be a focus of future research. Neither intervention improved osteoporosis self-efficacy, either at 2-years or 12-years. Therefore, more frequent osteoporosis education and bone density feedback is necessary to maintain knowledge and new interventions may be required to improve self-efficacy.

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Chapter 5: The effect of feedback of fracture risk and educational interventions on osteoporotic preventative behaviours and bone mineral density in premenopausal women

5.1 Introduction

Fractures are a major public health problem and can result in lower quality of life[1], disability and increased mortality rates[2]. Low bone mineral density (BMD) is a major contributor to fracture risk[3]. BMD in later life depends on the amount of bone gained in early life (peak bone mass) and the rate of subsequent bone loss[4]. There is significant age-related bone loss in premenopausal women[5] and premenopausal low bone mass is as important as fast rate of bone loss for the risk of fracture in later life[6]. Therefore, long-term maintenance or even improvement (for women whose peak bone mass is still accruing) in BMD in younger women is critical to preventing fractures in later life, and strategies to address this need to be identified.

Cigarette smoking[7], low levels of physical activity[8], and inadequate calcium intake[9] are well-accepted modifiable risk factors for low BMD. However, as osteoporotic fractures predominantly occur in later life, for early life interventions targeting these behaviours to be effective, they must result in persistent and/or ongoing improvements in behaviour and bone density. Research on how to make these changes is scarce in premenopausal women. One potential approach is to use individualised risk feedback[10], where information on fracture risk is provided

directly to participants. Previous controlled trials examining the effect of providing feedback of BMD screening in combination with osteoporosis education[11-13] suggest that women informed of having low BMD and/or higher fracture risk improve osteoporosis preventive behaviours. Our previous 2-yr randomised controlled trial aimed to determine the effects of individualised BMD feedback (normal vs. high fracture risk defined by mean spine and hip T-score) and two different educational interventions (the Osteoporosis Prevention and Self-management course (OPSMC) vs. an osteoporosis information leaflet) on osteoporosis preventive behaviours and BMD in premenopausal women[11]. In addition to favourable effects on behaviour, there were greater increases in femoral neck BMD in women receiving feedback of being at high fracture risk after 2 years of follow-up.

To our knowledge, there are no published trials with long-term follow-up (in excess of 2 years) of the effect of providing individualised information on fracture risk on behaviours likely to change fracture risk, or of BMD. Therefore, the aim of this study was to perform a further 10-yr follow-up of our previous 2-yr trial to examine the long-term effects of the feedback of fracture risk (high risk vs. normal risk) and educational interventions (OPSMC vs. an information leaflet) on BMD of femoral neck and lumbar spine as well as osteoporosis preventive behaviours.

5.2 Methods

5.2.1 Study population

This was an additional 10-yr follow-up of a registered (NCT00273260) 2-yr parallel randomised controlled trial previously conducted in 2000 in Southern Tasmania,

Australia, and the methods of which have been described in detail[11]. Women aged 25-44 years were randomly selected from the 2000 Tasmanian Electoral Roll and recruited between April and November 2000. As previously reported the population of southern Tasmania was predominantly Caucasian[11]. Women were excluded if they had previous measurement of bone density, thyroid disease, renal failure, malignancy, or rheumatoid arthritis, a history of hysterectomy or were taking hormone replacement therapies, were pregnant or planning pregnancy within 2 years of study entry, or were lactating. Ethics approval was obtained from Royal Hobart Hospital Ethics Committee and all participants gave written informed consent.

5.2.2 Intervention

Osteoporosis education

At baseline, 470 women were randomised 1:1 to receive one of two osteoporosis educational interventions: the OPSMC (OPSMC group, n=219) or an information leaflet (leaflet group, n=251). An independent statistician generated a list of random numbers as previously described [11]. Each participant was given a participant number on recruitment. While there was no allocation concealment, allocation was implemented sequentially for each participant number with no variations to the order in which the numbers were assigned. Randomisation was also performed prior to BMD results being known, so allocation could not be influenced by BMD status. The OPSMC is a chronic disease self-management course developed by the Arthritis Foundation of Victoria and utilized by Osteoporosis Australia. It aims to increase knowledge, improve confidence and awareness and self-management of osteoporosis

prevention with an emphasis on promoting appropriate lifestyle changes. OPSMC sessions of 2 hours were held weekly for 4 weeks with a maximum of 16 participants per group. The osteoporosis information leaflet, from Osteoporosis Australia "Understanding Osteoporosis", provided a comprehensive description of osteoporosis and a discussion of the role of lifestyle factors including diet, exercise and smoking, and optimal levels of calcium intake and exercise[12].

Feedback of fracture risk

Bone mineral density (BMD) was measured by dual-energy X-ray absorptiometry (DXA, Hologic QDR2000 densitometer, Waltham, MA) which was calibrated daily with coefficient of variation (CV) 1%. Measures were taken at the spine and hip at baseline, 2 years and 12 years by operators blinded to intervention status. At baseline, those with a mean spine and hip T-score <0 were informed that they were at a higher risk of fracture in later life (high risk group, n=232) whereas those with a mean T-score of 0 or greater were informed that they were not at higher risk (normal risk group, n=238). This was based on the observation that those in the lower half of the BMD distribution have threefold higher fracture risk both in later life and in the early postmenopausal period[14]. Although this cut-off is based on older women, it has been proposed that bone mass tracks throughout life as has been recorded in children[15], young[16], middle-aged, and aged populations[17], so that premenopausal women who are in the lower BMD range would still be expected to have lower BMD in the postmenopausal period. Participants in the leaflet information group received written feedback of fracture risk with their leaflet by mail, and those in OPSMC group received written feedback at the first session of the course. The comparison for the

fracture risk feedback component of the intervention is between women informed they were at higher risk (T-score <0) vs those who were not (T-score ≥ 0).

5.2.3 Outcomes

Primary outcomes for the 12-year follow-up were BMD at the femoral neck and lumbar spine; and calcium intake and calcium supplement use, and physical activity. These were measured yearly for first 2 years and again at 12 years as described below. Secondary outcomes were use of vitamin D supplements and smoking status.

Calcium intake was assessed by a short food frequency questionnaire (FFQ), which has been validated against 4 day weighed records and correlates well for estimated calcium intake (r=0.79, p=0.001)[18]. The calcium content of food categories was determined by the same Australian food composition tables[19] at each time point. This FFQ also assessed calcium supplement use. Participants were classified as taking calcium supplements if they reported taking a supplement containing calcium alone or as a main ingredient, and at a frequency of ≥ 4 times weekly.

Physical activity was assessed by a questionnaire validated in American adolescents[20], which we modified for Tasmanian conditions and had used previously in women of this age [21]. This questionnaire, asked how many days in the last 14 the participants reported performing at least 20 minutes of strenuous exercise and light exercise, measured in five categories (1 = 0 days, 2 = 1-2 days, 3 = 3-5 days, 4 = 6-8 days, 5 = 9 or more days).

Smoking status was assessed by questionnaire at baseline, 2 and 12 years. Participants were asked whether they were regular smokers, defined as smoking at least 7 cigarettes, cigars or pipes every week for at least 3 months.

Changes in osteoporosis preventive behaviours were determined by the status of behaviours at 2 and 12 years. Specifically, participants who smoked at 2 years but had quit smoking at 12 years were classified as ceased smoking, those who did not smoke at either 2 or 12 years as never smoking, those who did not smoke at 2 years but were smoking at 12 years as commenced smoking, and as persistent smoking otherwise. A similar classification was used for change in use of calcium supplements. Depending on the difference between 2 and 12 years, change in calcium intake was categorized as increased (higher at 12 years) or decreased (lower at 12 years) and change in physical activity was classified as unchanged, increased and decreased.

Use of vitamin D supplements was assessed at 12 years. Participants were asked to recall if they had regularly used vitamin D supplement at each year during the last 12 years. Regular use was defined as taking supplements at least 5 times per week for more than 9 months of the year. Participants were categorised into 2 groups: recent use if using vitamin D supplements for the preceding 2 consecutive years and no recent use otherwise.

5.2.4 Other study factors

Serum 25-hydroxy vitamin D (25(OH)D) level was assessed at 12 years, from venous blood samples, using liquid chromatography (LC)-tandem mass spectrometry (MS) (CV 3-6%, using an internal standard).

Other study factors measured at baseline, 2 years and 12 years included height by stadiometer (The Leicester height measure, Invicta Plastics Ltd, Oadby, England) and weight by a single set of calibrated scales (Heine, Dover NH USA). Body mass index was calculated [weight (kg)/height^2 (m^2)]. Breastfeeding history, number of children, family history of osteoporosis and/or fracture education level, employment status of main financial provider in the household, and marital status were assessed by questionnaire at baseline, 2 years and 12 years. Self-reported fractures with age at each fracture and menopausal status was also reported at 12 years. Date of fracture was estimated from age at fracture. A women reporting at least one fracture occurring after the date of the baseline assessment was considered to have sustained an incident fracture.

5.2.5 Statistical analysis

The sample size was calculated for the original study, as described previously[11]. All analyses were based on original assigned groups.

Mean (SD) and number (%) were used to describe continuous and categorical variables, respectively. Linear mixed-effects modelling was used to estimate the effects of the feedback of fracture risk and the educational intervention on absolute changes in FN and LS BMD from 2 to 12 years, with adjustment for potential confounders. Intervention groups (educational intervention group and fracture risk feedback group), time (coded as a binary variable to denote follow-up number) and the interaction between the interventions and time (treatment effect, i.e., group by time in Table 5.2) were entered as fixed factors in the model. Participant identification

number was included as a random effect to account for the dependence of repeated observations. Log binomial and log multinomial regression models were used to estimate the relative risk (RR) of categories of behaviour change, from 2 to 12 years, associated with the fracture risk feedback and educational interventions. Models were further adjusted for age, anthropomorphic and sociodemographic factors, and menopausal status at the 12 year follow up. Covariates were retained in the model when the estimated coefficient for the intervention effect changed by more than 10%. To handle missing data, we assumed data were missing at random and used a weighted estimating equation method[22,23]; we estimated the probability of an outcome being observed by fitting a logistic regression model using the baseline characteristics age, education level and smoking status, for which complete data were available. In subsequent analyses, complete cases were weighted by the inverse of their estimated probabilities of being observed. All analyses were performed in Stata version 12 (Stata Corporation, Texas, USA). A two-tailed p value <0.05 was considered statistically significant.

5.3 Results

Participant characteristics

A total of 470 women (64% response rate) aged 25 to 44 years were recruited at baseline with 94% (n=415) retained at year 2 and 74% (n=347) at year 12 (Figure 5.1). Baseline characteristics between women who did and did not reach 12-yr follow-up were compared (

Supplemental Table 5-1**)**. Women who completed 12-yr follow-up had similar characteristics to those who were lost to follow-up, other than being slightly (2 years) older.

Table 5.1 shows the characteristics for participants at baseline: women in the high risk group were shorter and lighter and, as expected, had lower femoral neck (FN) and lumbar spine (LS) BMD than women in the normal risk group; there was a greater proportion of women who had ever smoked among those who received the information leaflet than among those who received the OPSMC. Menopausal status at 12 years was similar across the groups as were other baseline characteristics. Serum 25(OH)D level at 12 years was slightly higher in the high risk group than the normal risk group but was similar between educational intervention groups. Self-reported fracture numbers were similar between educational and fracture risk feedback groups.

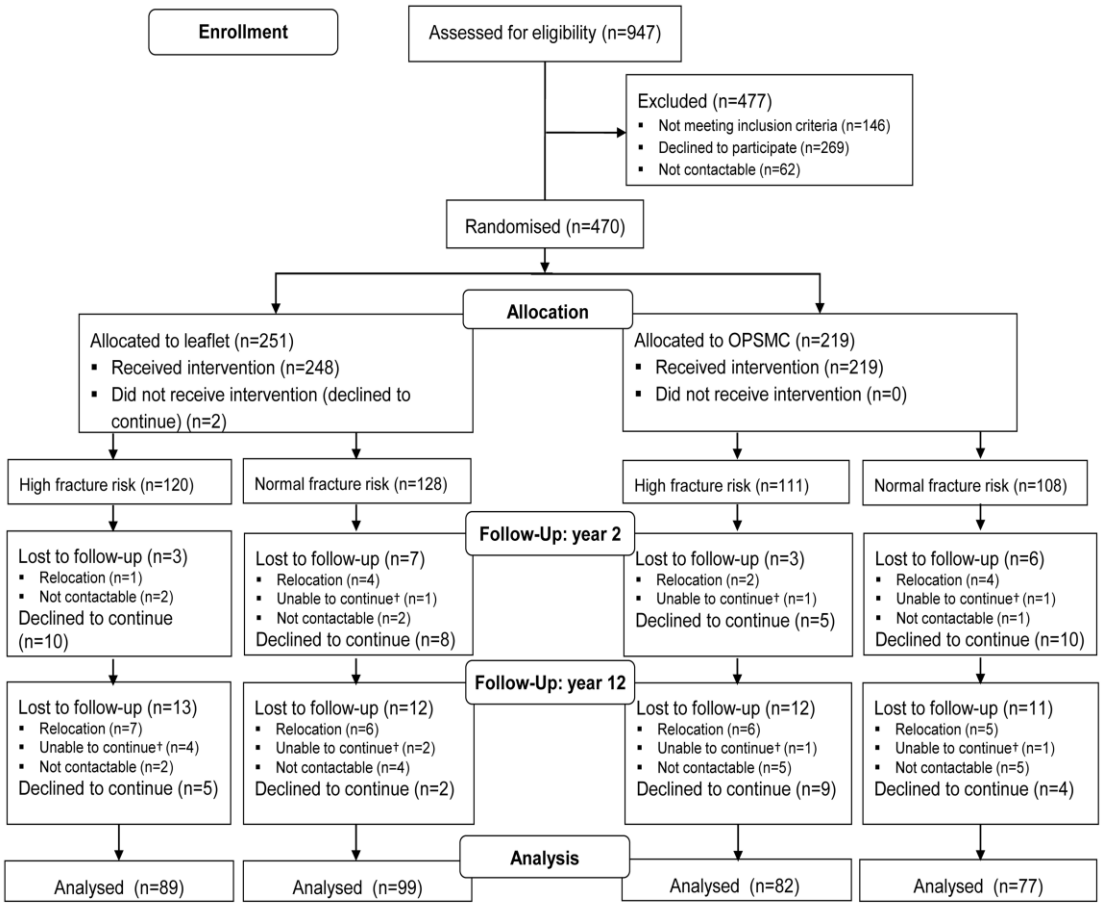

Figure 5.1: Flow diagram of study population. OPSMC=Osteoporosis Prevention and Self-management course. †Unable to continue due to health related issues, including illness, pregnancy, disability and mortality

Supplemental Table 5-1: Comparison of baseline characteristics of participants who did and did not complete the study

Characteristic	Completed study	Withdrawals	P-value
	n= 347	n= 120	
Age (yr.)	38.3 (5.2)	36.3 (5.6)	<0.001
Feedback of high fracture risk n	177 (51)	60 (50)	0.892
Received OPSMC n (%)	160 (46)	60 (50)	0.462
Height (cm)	163.5 (6.3)	162.1 (6.6)	0.084
Weight (kg)	69.6 (13.4)	69.3 (14.3)	0.819
Strenuous activity level (Median)	3	3	0.345
Calcium intake (mg/d)	782.3 (401.5)	808.6 (391.2)	0.533
Calcium supplement use n (%)	7 (2.0)	3 (2.5)	0.414
BMD of FN (g/cm^2)	0.93 (0.13)	0.93 (0.15)	0.797
BMD of LS (g/cm^2)	1.08 (0.12)	1.08 (0.12)	0.914

All values are mean (SD) unless otherwise indicated.
OPSMC=Osteoporosis Prevention and Self-management course.
BMD=bone mineral density.

Table 5.1: Characteristics of participants by fracture risk group, and by education intervention group

	Fracture risk group		Educational intervention	
	High	Normal	OPSMC	Leaflet
Baseline	n=231	n=236	n=220	n=247
Age (years)	37.9 (5.2)	37.6 (5.5)	37.4 (5.5)	38.1 (5.2)
Height (cm)	162.0 (6.5)	164.2 (6.0)	162.7 (6.3)	163.5 (6.5)
Weight (kg)	64.4 (10.3)	74.6 (14.5)	69.3 (13.6)	69.7 (13.6)
Education level [n (%)]				
≤Grade 10	76 (33)	79 (34)	76 (35)	79 (32)
Grade 10 to 12	50 (22)	49 (21)	39 (18)	60 (24)
>Grade 12	105 (45)	106 (45)	104 (47)	107 (44)
Employment status [n (%)]				
0 h/week	32 (14)	32 (14)	27 (12)	37 (15)
≤ 20 h/week	56 (24)	53 (22)	51 (23)	58 (24)
> 20 h/week	142 (62)	151 (64)	142 (65)	151 (61)
Currently smoking [n (%)]	37 (16)	42 (18)	32 (15)	47 (19)
Ever smoked [n (%)]	113 (49)	113 (48)	94 (43)	132 (54)
Married or de facto [n (%)]	167 (72)	173 (73)	169 (77)	171 (69)
Calcium intake (mg/d)	777 (379)	801 (418)	799 (397)	780 (401)
Strenuous activity level (median)	3	3	3	3
12-years	n=171	n=176	n=160	n=187
Menopause status [n (%)]				
Post menopause	45 (26)	41 (23)	47 (29)	39 (21)
Pre-menopause	65 (38)	69 (39)	61 (38)	61 (38)
Status unclear	10 (6)	16 (9)	14 (9)	14 (9)
Currently menopausal	51 (30)	50 (29)	38 (24)	38 (24)
Serum 25(OH)D levels (nmol/L)	65.5 (23.2)	60.9 (22.3)	64.0 (23.5)	64.0 (23.5)

All values are Mean (SD) unless otherwise indicated.
OPSMC = Osteoporosis Prevention and Self-management course.
BMD = bone mineral density.
Bold denotes statistical significant.

Changes in BMD and effects of interventions

Unadjusted FN and LS BMD at each time point (stratified by fracture risk feedback group and educational intervention group) are shown in Supplemental Table 5-1.

Table 5.2 shows the mean (95% CI) change in BMD between 2 and 12 years according to fracture risk feedback and educational intervention, along with the estimated effects of the interventions over time from the linear mixed model analyses. There were 325 women who had BMD measured at both 2 and 12 years. Both FN and LS BMD were lower at 12 years than at 2 years in both fracture risk feedback and both educational intervention groups as well as in the study sample as a whole ($p< 0.001$ for all). We found evidence for a smaller reduction in FN BMD between 2 and 12 years for women who received feedback of high fracture risk compared to those who received feedback of normal fracture risk, and this difference persisted after adjusting for potential confounders ($\beta=0.023$, 95% CI=0.005 to 0.041), but no evidence for a similar effect for LS BMD ($\beta=-0.011$, 95% CI=-0.027 to 0.006). We found no evidence for interaction between educational intervention and time (treatment effect) or interaction between fracture risk feedback and educational intervention for either FN or LS BMD.

Supplemental Table 5-2: Unadjusted FN and LS BMD at each time point (stratified by fracture risk feedback group and educational intervention group)

	High fracture risk	Normal fracture risk
	(n=231)	(n=236)
FN BMD baseline	0.836 (0.823, 0.850)	1.018 (1.005, 1.030)
LS BMD baseline	0.992 (0.982, 1.002)	1.169 (1.157, 1.181)
	(n=213)	(n=206)
FN BMD 2-yr	0.859 (0.848, 0.870)	1.033 (1.019, 1.046)
LS BMD 2-yr	0.994 (0.983, 1.004)	1.172 (1.158, 1.185)
	(n=171)	(n=176)
FN BMD 12-yr	0.736 (0.723, 0.749)	0.889 (0.873, 0.905)
LS BMD 12-yr	0.936 (0.920, 0.953)	1.130 (1.111, 1.149)

	OPSMC	Leaflet
	(n=220)	(n=247)
FN BMD baseline	0.934 (0.915, 0.954)	0.923 (0.907, 0.938)
LS BMD baseline	1.084 (1.066, 1.101)	1.080 (1.065, 1.094)
	(n=197)	(n=222)
FN BMD 2-yr	0.945 (0.927, 0.964)	0.944 (0.928, 0.959)
LS BMD 2-yr	1.079 (1.061, 1.098)	1.083 (1.067, 1.099)
	(n=160)	(n=187)
FN BMD 12-yr	0.813 (0.795, 0.832)	0.814 (0.795, 0.832)
LS BMD 12-yr	1.030 (1.007, 1.054)	1.038 (1.017, 1.060)

Values are mean (95% confidence interval).

Table 5.2: Absolute change in BMD in each intervention group and effect of fracture risk feedback and educational interventions on absolute change in BMD between 2 and 12 years

	Mean of change [a] (95% CI)	**Unadjusted β (95% CI)**	**Adjusted [b] β (95% CI)**
		Group by time	**Group by time**
Femoral neck (g/cm^2)			
Normal risk (n=162)[d]	**-0.145 (-0.159, -0.132)**	reference	reference
High risk (n=163)[c]	**-0.123 (-0.134, -0.112)**	**0.022 (0.005, 0.040)**	**0.023 (0.005, 0.042)**
Leaflet (n=176)[d]	**-0.130 (-0.143, -0.118)**	reference	reference
OPSMC (n=149)[c]	**-0.139 (-0.152, -0. 127)**	-0.009 (-0.026, 0.009)	-0.011 (-0.029, 0.008)
Lumbar spine (g/cm^2)			
Normal risk (n=162)[d]	**-0.041 (-0.052, -0.029)**	reference	reference
High risk (n=163)[c]	**-0.055 (-0.066, -0.044)**	-0.011 (-0.027, 0.005)	-0.011 (-0.027, 0.006)
Leaflet (n=176)[d]	**-0.047 (-0.058, -0.036)**	reference	reference
OPSMC (n=149)[c]	**-0.048 (-0.060, -0.036)**	0.001 (-0.016, 0.017)	0.002 (-0.015, 0.018)

OPSMC=Osteoporosis Prevention and Self-management course.
CI=confidence interval.
Linear mixed-effects model was used to test treatment effect (group by time).
[a] Unadjusted absolute change in BMD from 2 to 12 years within each subgroup.
[b] Adjusted for other items in column, duration of follow-up, age at 2 years, change in weight and height between 2 years and 12 years and menopause status at 12-year.
[c] Intervention group.
[d] Control group.
Bold denotes statistical significant.

Table 5.3: The effect of fracture risk groups and educational intervention on the change in behaviours between 2 and 12 years

	Fracture risk group		**RR (95% CI)**		**Educational intervention**		**RR (95% CI)**	
	High	**Normal**	Unadjusted	Adjusted†	**OPSMC**	**Leaflet**	Unadjusted	Adjusted†
Smoking	n=151	n=144			n=132	n=163		
Never smoked	133 (88)	121 (84.0)	1.00	1.00	116 (88)	138 (85)	1.00	1.00
Cessation	12 (8)	6 (4.2)	1.91 (0.74, 4.95)	1.85 (0.70, 4.89)	12 (9)	6 (4)	2.47 (0.95, 6.40)	2.27 (0.86, 6.01)
Commenced or persistent smoking	6 (4)	17 (11.8)	**0.34 (0.14, 0.83)**	**0.33(0.13, 0.80)**	4 (3)	19 (12)	**0.26 (0.09, 0.75)**	**0.28 (0.10, 0.79)**
Calcium intake	n=162	n=161			n=149	n=174		
Decreased	66 (41)	72 (45)	1.00	1.00	67 (45)	71 (41)	1.00	1.00
Increased	96 (59)	89 (55)	0.91 (0.71, 1.17)	0.89 (0.69, 1.15)	82 (55)	103 (59)	1.10 (0.86, 1.42)	1.15 (0.89, 1.48)
Calcium supplements	n=161	n=161			n=149	n=173		
Never supplement	79 (49)	112 (70)	1.00	1.00	87 (59)	104 (60)	1.00	1.00
Commenced or persistent supplement	74 (46)	44 (27)	**1.68 (1.24, 2.28)**	**1.66 (1.22, 2.24)**	57 (38)	61 (35)	1.08 (0.81, 1.45)	1.12 (0.83, 1.50)
Cessation	8 (5)	5 (3)	1.60 (0.53, 4.79)	1.52 (0.50, 4.59)	5 (3)	8 (5)	0.73 (0.24, 2.17)	0.62 (0.21, 1.87)
Vitamin D supplements‡	n=171	n=175			n=159	n=187		
No recent use	122 (71)	150 (86)	1.00	1.00	119 (75)	153 (82)	1.00	1.00
Recent use	49 (29)	25 (14)	**2.01 (1.30, 3.09)**	**1.99 (1.27, 3.11)**	40 (25)	34 (18)	1.38 (0.92, 2.08)	1.37 (0.90, 2.09)
Strenuous Physical activity	n=162	n=161			n=149	n=174		
Unchanged	56 (35)	49 (30)	1.00	1.00	42 (28)	63 (36)	1.00	1.00
Increased	43 (26)	50 (31)	0.85 (0.61, 1.21)	0.90 (0.62, 1.31)	40 (27)	53 (31)	0.88 (0.62, 1.25)	0.87 (0.62, 1.24)
Decreased	63 (39)	62 (39)	1.01 (0.77, 1.33)	1.02 (0.77, 1.37)	67 (45)	58 (33)	**1.35 (1.02, 1.78)**	1.30 (0.99, 1.71)
Light physical activity	n=161	n=161			n=148	n=174		
Unchanged	75 (47)	64 (40)	1.00	1.00	59 (40)	80 (46)	1.00	1.00
Increased	47 (29)	43 (2)	1.09 (0.77, 1.55)	1.13 (0.80, 1.59)	44 (30)	46 (26)	1.12 (0.79, 1.60)	1.07 (0.76, 1.52)
Decreased	39 (24)	54 (33)	0.72 (0.51, 1.02)	**0.71 (0.51, 0.99)**	45 (30)	48 (28)	1.10 (0.78, 1.55)	1.16 (0.84, 1.61)

Values are n (%) unless otherwise indicated. RR, relative risk; CI, confidence interval; OPSMC=Osteoporosis Prevention and Self-management course.
Log binomial and multinomial regression models were used as appropriate.
†Adjusted for age at 2 years, baseline number of children, employment status, education level and marital status.
‡ recent use if using vitamin D supplements for the preceding 2 consecutive years and no recent use otherwise. See content for the groupings of all behaviours in detail.
Bold denotes statistical significant.

Effects of interventions on osteoporosis preventive behaviours

Table 5.3 gives the estimated relative risk of each category behaviour change from 2 to 12 years between fracture risk feedback groups and between educational intervention groups. Compared to women with normal fracture risk, those with high fracture risk were more likely to have ceased smoking than never smoked (relative risk (RR) = 1.85, 95% CI = 0.70 to 4.89) and less likely to have commenced smoking or persistently smoked than never smoked (RR = 0.33, 95% CI = 0.13 to 0.80). Compared to those in the normal fracture risk group, women in the high fracture risk group were more likely to commence or keep using calcium supplements (compared to never using supplements; RR = 1.66, 95% CI = 1.22 to 2.24), and to report using vitamin D supplements for the preceding two consecutive years (RR = 1.99, 95% CI = 1.27 to 3.11). They were also less likely to report decreased than unchanged light physical activity (RR = 0.71, 95% CI = 0.51 to 0.99) than those with normal risk. The OPSMC group had a more favourable pattern of smoking behaviour change compared to the leaflet group (RR = 2.27, 95% CI = 0.86 to 6.01 for smoking cessation; RR = 0.28, 95% CI = 0.10 to 0.79 for commenced or persistent smoking relative to never smoked group). No differences between educational intervention groups were observed for use of either calcium or vitamin D supplements or change in light physical activity. There were no between group differences in the probability of changing dietary calcium intake or strenuous physical activity. Serum 25(OH)D levels were higher in participants reporting vitamin D supplement use for the preceding 2 consecutive years (mean (SD) 76.6 (23.8) vs. 59.4 (21.1) nmol/L, $p < 0.001$).

5.4 Discussion

This 10-yr follow-up of a 2-yr randomised controlled trial in premenopausal women is, as far as we are aware, the first to evaluate the long-term effects of feedback of high fracture risk and group education on change in BMD and osteoporosis preventive behaviours. The feedback of high fracture risk was associated with a slower loss of FN BMD but not LS BMD, improved use of calcium and vitamin D supplements and a favourable effect on smoking status. The OPSMC was associated with improved smoking behaviour compared to a leaflet but not with additional benefits for BMD. These changes suggest that feedback of bone density testing results with an assessment of relative fracture risk could be considered in young women as a strategy to improve long-term bone health and prevent osteoporosis in later life. Furthermore, the improvements in behaviours may be beneficial for other health issues suggesting an approach targeting bone may have wider benefits.

Previous studies have demonstrated the short-term (up to 2 years) benefits of the feedback of fracture risk or BMD results for improving osteoporosis preventive behaviours, for example, calcium supplement use as well as increased physical activity among young women[11-13,24]. In the first two years of follow-up of our study[11] , women receiving feedback of high fracture risk had a significantly greater increase in FN BMD than those in the normal risk group, and improved calcium supplement use and self-reported physical activity at two years. In comparison, the present study focused on long-term effects, showing that the short-term benefits for calcium supplements use and FN BMD [11] persisted after a subsequent 10 years follow-up, but improvements in self-reported physical activity did not.

In addition, the longer follow-up period enabled us to detect effects on smoking behaviour with the probability of women quitting smoking in the high risk being around double that of those in the normal risk group. Women in this group were also 67% less likely to commence or have been persistently smoking by 12 years. These are substantial effects – they are greater than cessation rates achieved by print-based self-help interventions[25] and telephone counselling[26]. Similar effects of education intervention were found for changes in smoking behaviours. Such improvements have potential benefits for prevention of a wide range of diseases other than osteoporosis. Smoking is the leading preventable cause of mortality including atherosclerotic cardiovascular disease, lung cancer, and chronic obstructive pulmonary disease (COPD) – the three major causes of smoking-related mortality[27]. The fact that there was no effect on smoking cessation at 2 years is unsurprising, as smoking cessation was not a primary outcome of the 2 year study and the longer period of follow-up allowed for accumulation of the effect of the intervention over time, making it possible to detect differences in smoking behaviour at 12 years. The feedback of high fracture risk was also associated with use of vitamin D supplementation in the long-term, with the probability of participants using vitamin D supplement consecutively for the preceding 2 years in the high risk group being double that of the normal risk group. The intervention was associated with a slowing of FN BMD loss equivalent to about 2.4% over 10 years. It has been estimated that for each 5% loss in FN BMD in elderly women there is a 40% and 90% increase in all fractures and hip fracture risk, respectively[28]. Thus, slowing FN BMD loss by 2.4% is likely to be important for the prevention of osteoporosis and fracture in later life.

We observed no effect of feedback of high fracture risk on LS BMD which is unsurprising as there was also no such effect after 2 years[11]. This could be explained by the possibility of site-specific responses of bone to lifestyle behaviour changes, such as physical activity. Such site specificity has been demonstrated for exercises in premenopausal women, with BMD of lumbar spine but not femoral neck, total hip or whole body being improved by lower and upper body resistance plus jump exercise, compared to lower body resistance plus jump exercise or no intervention control[29]. Randomised controlled trials of calcium supplements in younger women have also shown variations in effects at different sites, though the reasons for this are unclear[30].

Given the lack of effect of the OPSMC on either behaviour or BMD at 2 years[11], it was not surprising that the 10-year follow-up data also failed to find any additional effect of the OPSMC on change in BMD in comparison to an information leaflet intervention, although interestingly, it was associated with long-term smoking behaviour. Importantly, this effect is unlikely to be explained by any confounders, as women were randomised to either group at baseline. As we stated in our previous study[11], the OPSMC was designed similarly to a chronic disease self-management course for arthritis, which has been shown to have only a small effect on improving health status and behaviours even in symptomatic populations[31,32]. However, our study was conducted in healthy women, who may be less motivated to change than those having a symptomatic condition. This lack of effect of the OPSMC for BMD and most behaviours is consistent with our finding that the effect of the OPSMC on osteoporosis knowledge was greatest at 6 weeks, persisted at a lower level at 2

years[33] but was no longer significant at 12 years[34]. Why the OPSMC would influence smoking but not other behaviours in such a population is unclear.

This study has several potential limitations. The 64% response rate may have resulted in selection bias, but as previously discussed [11], although this sample had a lower proportion of current smokers (17%) compared to the Tasmanian prevalence of daily smoking (29%) in women aged 25 to 44 years in 1998, socioeconomic factors like educational levels and the unemployment rate in our study approximate the overall population figures. Therefore, while this sample may be not fully representative of the Tasmanian population and the generalizability of our study findings to other racial/ethnic populations is uncertain, they are likely to be generalisable to healthy Caucasian women in this age range given that the population of the region was predominantly Caucasian at study entry. Missing data due to drop-outs is another potential limitation, and women lost to follow-up were younger. However, we took this potential bias into account by using the combination of linear mixed-effects model and inverse probability weighting. The results were similar whether this method or analysis by using only complete case data was used, so the likelihood of loss to follow-up influencing our findings is low. Although feedback of fracture risk was not randomised, we adjusted for potential confounders to minimise the risk of bias. In comparison, the randomised nature of the trial of educational intervention provides the strongest evidence for the OPSMC effect to have caused the smoking behaviour change. Blinding is an inevitable issue given that participants were not able to be blinded to interventions; however, the measurement of BMD, the most clinically important outcome in our study, was measured by DXA, by an operator blinded to the

status of interventions. This objectively measured outcome would be unlikely to be biased by subjective factors. Self-reported behavioural measures could possibly be influenced, but the fact that objectively measured serum 25(OH)D concentrations at 12 years were significantly and substantially higher in women who reported recent vitamin D supplement at 12 years supports the validity of our self-report data, as do previous studies validating self-reported smoking[35,36]. It was considered unethical to perform DXA but withhold participants' results so we are unable to compare any effects of fracture risk feedback with a no feedback control group. BMD changes form behavioural changes may not persist if the behaviour changes made do not persist. However, as there are no data of which we aware examining the effects of reversing 10 years of beneficial behaviours on BMD, it is unclear the magnitude any deteriorations in behaviour in the future might have. Moreover, given the 12 years of persistent improvement we have demonstrated, it seems unlikely in any case that a rapid reversal of all positive behaviours would be likely in the future. Finally, fractures were not included as a primary or secondary outcome. The sample size for the original two-year RCT was based on BMD and osteoporosis preventive behaviour endpoints rather than fracture because of the very short-term follow-up and younger age of study participants and the power of long-term study to assess fracture endpoints was also limited.

Conclusions

In conclusion, feedback of high fracture risk was associated with long-term benefits for improving calcium and vitamin D supplement use and possibly smoking behaviour, as well as slowing loss of FN but not LS BMD in premenopausal women.

The OPSMC was associated with improved smoking behaviour but not a slower decrease in BMD of either FN or LS. Bone density feedback with an assessment of fracture risk could be considered in young women as a strategy to improve long-term bone health and prevent osteoporosis in later life. Furthermore, its resulting improvements in behaviours may be beneficial for other health issues beyond bone suggesting an approach targeting bone may have wider benefits. However, the cost-effectiveness of implementing a population-based osteoporosis prevention strategy based on DXA screening requires assessment.

5.5 References

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Chapter 6: Cut-points for associations between vitamin D status and multiple musculoskeletal outcomes in middle-aged women

6.1 Introduction

Bone mineral density (BMD)[1,2] starts to decline in the premenopausal period and premenopausal low bone mass is as important as fast rate of bone loss for the risk of fracture in later life[3]. Equally important, impaired balance and mobility increases risk of falls[4], another important contributor to fracture risk, with 4-39% of falls in people older than 65 years accounted for by gait/balance disorders[5]. Importantly, balance begins attenuating after 45-55 years of age[6,7], particularly in women[8]. Moreover, muscle weakness is an important contributor to decreased balance and functional limitations in older people[9,10]. Similarly, muscle mass and strength may also have an accelerating decline in middle-aged women[11], and the reason for this is multifactorial, such decreased androgen concentrations, reduced physical activity level, deficient nutrients, chronic inflammation, and insulin resistance[12]. Therefore, it is critical to maintain or even improve BMD, muscle strength and balance in middle-aged people in the effort to reduce the risk of functional limitations, falls and fractures in older age.

The important role of vitamin D in bone and mineral homeostasis is well-established. Studies also suggest that vitamin D status is associated with muscle weakness[13-15], balance[15] and falls[16]. However, there is controversy concerning the optimal level of

serum 25-hydroxyvitamin D (25(OH)D) for musculoskeletal health, particularly in younger people. The level of serum 25(OH)D which maximally suppresses serum PTH has been most commonly used to define the optimal 25(OH)D level, but these estimates have a wide range from 25 to 122 nmol/L[17,18]. While PTH is associated with increased bone loss, this approach does have limitations. Levels of PTH fluctuate widely, varying with diet, physical activity, time of day[19] and season[20]. Also, studies have shown a high prevalence of functional hypoparathyroidism in the elderly, i.e., the absence of secondary hyperparathyroidism in the presence of hypovitaminosis D[21,22], which also makes the use of PTH problematic. Furthermore, a recent study in older adults suggested that thresholds for serum 25(OH)D may differ by different outcomes (e.g., grip strength, falls, physical performance and fractures)[23] but as far as we are aware, there is limited information on the optimal level of serum 25(OH)D concentration for musculoskeletal outcomes in middle-aged women. Most studies include only a single musculoskeletal outcome and none examine muscle strength and balance in this age group.

Therefore, the main objectives of this cross-sectional analysis were to: a) determine whether there are identifiable cut-points of serum 25(OH)D for associations between serum 25(OH)D and multiple musculoskeletal outcomes in middle-aged women; and if so, b) whether below those identified cut-points greater 25(OH)D concentrations have beneficial associations with those outcomes and if above them no such beneficial associations exist.

6.2 Materials and Methods

6.2.1 Study sample

Participants were from a 10-years additional follow-up of 2-year randomized controlled trial conducted in 2000 in Southern Tasmania, Australia, details of which have been reported elsewhere[24]. At baseline, women aged 25-44 years were randomly selected from the 2000 Tasmanian Electoral Roll. Women were excluded if they had previous measurement of bone density; had medical conditions affecting BMD (thyroid disease, renal failure, malignancy, or rheumatoid arthritis); a history of hysterectomy or were taking hormone replacement therapies; or who were pregnant, planning pregnancy within 2 years of study entry, or lactating. At baseline, 470 women were randomly assigned to one of two osteoporosis educational interventions: group education using the Osteoporosis Prevention and Self-management course (OPSMC) or an information leaflet. Participants had their BMD measured at the spine and hip at baseline, 2 years and 12. At baseline, those with a mean spine and hip T-score <0 were informed that they were at a higher risk in later life whereas those with a mean T-score of 0 or greater were informed that they were not at higher risk. The present study is a cross-sectional analysis of the 344 women retained in the study and who had serum 25(OH)D levels measured (mean age of 50.0 years, 36.2 to 56.8 years of age) at 12 years. Ethics approval was obtained from the Tasmania Health and Medical Human Research Ethics Committee and all participants gave written informed consent.

6.2.2 Measurements

Serum 25(OH)D levels

Venous blood samples were taken with no requirement for fasting or time. Serum 25(OH)D was assayed by liquid chromatography tandem mass spectrometry (LC-MS/MS). The assay measures 25(OH)D2 and 25(OH)D3 separately with a CV 3-6%, using an internal standard.

BMD

BMD was measured at the lumbar spine (LS) and femoral neck (FN) by dual-energy X-ray absorptiometry (DXA) using fan beam setting on an in-house Hologic Delphi bone densitometer (Hologic QDR2000, Waltham, MA), calibrated daily with coefficient of variation (CV) 1%.

Balance measurements

Balance was assessed using 4 clinical balance tests - the timed up and go test (TUG), the step test (ST), the functional reach test (FRT) and the lateral reach test (LRT). All have been validated in older women and have normative values determined in women of the age in our study[25].

The TUG requires participants to sit in a normal armchair (45 cm high) with their back against the chair. They were timed when standing up, walking a distance of 3 m, turning around, walking back and sitting down with back against chair again. The average time of two trials was used for analysis. The test is reliable (intraclass

correlation coefficient (ICC) = 0.99 for both interrater and retest reliability)[25] and is strongly associated with falls risk in older adults [26].

The ST measures speed of performing a dynamic standing task. Participants stood on one leg 5 cm from an 8.5-cm-high block positioned against a wall and placed the whole foot of the other leg onto the block and then returned it to the floor repeatedly as fast as possible for 15 seconds. The number of steps was recorded. Both sides were tested, and the mean number of steps for each side was calculated for analysis. The ST has a high reliability (ICCs > 0.90 in healthy older people)[27].

The FRT measures ability to reach forward with each arm from a bilateral stance position[28]. Participants stood with feet a comfortable distance apart behind a line perpendicular and adjacent to a wall. The arm closest to the wall was raised to shoulder height and the position of the knuckle of the middle finger measured [28]. Keeping the feet flat they leaned forward as far as possible and the position of the knuckle was recorded at the point of furthest reach. FRT was the difference between the two measures. The mean score of three trials for each side was calculated for analysis. This test has a high interrater reliability (ICC = 0.98)[28].

The LRT measures ability to reach to the side in bilateral stance[29]. Participants stood with their backs near but not touching a wall with the heels 10 cm apart. Participants raised both arms to shoulder height and maintained equal weight bearing while the position of the third finger's tip on the side being measured was marked on the wall. Participants then lowered the arm not being measured and reach sideways as far as possible with the arm being measured. The position of furthest reach was marked and

the difference between the two marks calculated. The mean of the three trials on each side was calculated for analysis. LRT has a high retest reliability (ICC > 0.94) in healthy older women[29].

Lower limb muscle strength (LMS)

LMS was measured to the nearest kilogram using a dynamometer (TTM Muscular Meter, Tokyo, Japan) [30]. This test examines isometric strength, predominantly of the quadriceps and hip extensors. The examiner demonstrated the correct technique to the participant before testing. Participants stood on the back of the dynamometer platform, with back straight against a wall and knees flexed to an angle of 115 °. They held a bar, connected to the dynamometer by a chain, and lifted the bar using maximum force using their legs, with the back and neck straight. Two readings were made, and the mean calculated for analysis. The intraclass correlation coefficient for LMS was 0.94 (95%CI, 0.93, 0.95) in this study.

Dietary intake

Usual food intake was estimated using a food frequency questionnaire (Anti-Cancer Council of Victoria), which has been validated against 7-day food diaries with energy-adjusted correlation coefficients for nutrient intakes ranging from 0.28 for vitamin A to 0.78 for carbohydrate[31]. Intakes of calcium, energy, fat, protein, carbohydrate, cholesterol, iron, magnesium, phosphorus, sodium, vitamin C, vitamin E, and zinc were calculated using NUTTAB 2010. The content of calcium in various food categories was determined by Australian food composition tables[32]. Participants were also asked to recall if they had regularly used calcium and vitamin D supplements during the last

year, where regular use means taking supplements at least 5 times per week for more than 9 months of the year.

Physical activity

We measured physical activity using a validated questionnaire[33], which was modified for Tasmanian conditions and used previously in women of this age, where physical activity was related to bone mass of the femoral neck [34]. This questionnaire assessed strenuous and light physical activity levels by asking participants how many days in the last 2 weeks they reported performing at least 20 minutes of strenuous exercise and light exercise, represented by five categories (1 = 0 days, 2 = 1-2 days, 3 = 3-5 days, 4 = 6-8 days, 5 = 9 or more days).

Anthropometry and other factors

Height was measured by stadiometer (The Leicester height measure, Invicta Plastics Ltd, Oadby, England), weight by a single set of calibrated scales (Heine, Dover NH USA) and body mass index (BMI) (calculated weight (kg)/height $(m)^2$). Questionnaire assessment was made of smoking history (current/former/never), breastfeeding history, number of children, family history of osteoporosis and/or fracture, and fracture history in the subject, education level, employment status of main financial provider in the household, menopausal status, and marital status.

6.2.3 Statistical analysis

To adjust for the seasonal variation of 25(OH)D, deseasonalized vitamin D levels were calculated by regressing the measured 25(OH)D level on the sinusoidal function:

$\sin(2\pi[\text{day of year drawn}/365]) + \cos(2\pi[\text{day of year drawn}/365])$, and then adding the residuals to the average estimated 25(OH)D concentration to create a deseasonalized vitamin D level for each individual.

Participants' characteristics were presented using mean (SD) or number (%) as appropriate. Difference in characteristics between women with deseasonalized 25(OH)D level below and above 50 nmol/L were tested using Student's t-test or Kruskal-Wallis or Chi-square test as appropriate. Residuals of all outcomes predicted from regression models were approximately normally distributed, so no transformation was made.

To adjust for potential confounders, adjusted values were generated for each outcome by regressing each measured outcome on its specific confounding factors, and then adding the residuals to the mean of each measured outcome. Adjusted values for deseasonalized 25(OH)D levels were also generated in the same way using the same outcome specific covariates. The raw data and adjusted values were used for unadjusted and adjusted analyses, respectively. Both locally weighted (LOWESS) regression plots and nonlinear least-squares estimation were used to determine unadjusted and adjusted cut-points for associations of 25(OH)D with LS BMD, FN BMD, LMS, TUG, FRT, LRT and ST (Table 2). Segmented regression using adjusted values was further utilized to determine associations (beta coefficients) for participants with 25(OH)D below and above the identified adjusted cut-points (Table 3). All analyses were performed in Stata version 12 (Stata Corporation, Texas, USA). A two-tailed p value <0.05 was considered statistically significant.

6.3 Results

Baseline characteristics of participants who did and did not complete the 12 year follow-up have been previously reported[35]. Briefly, women lost to follow-up (26%) were younger, had lower levels of educational attainment, and were more likely to be current smokers or to have ever smoked, and less likely to be married or in a de facto relationship compared to those who were retained, but other anthropometric and demographic factors were comparable. Table 6.1 gives comparisons of characteristics of participants with deseasonalized 25(OH)D levels below and above 50 nmol/L. In comparison to women with deasonalised 25(OH)D <50 nmol/L, women with 25(OH)D of 50 nmol/L or more were less likely to be current smokers, had lower weight and BMI, higher strenuous physical activity level and proportion of vitamin D and calcium supplement use, but had lower lumbar spine (LS) BMD. The prevalence of low deseasonalized 25(OH)D was 6% (<30 nmol/L) and 28% (<50 nmol/L).

Table 6.1: Characteristics of participants (n=344)

	Total	Deseasonalized 25(OH)D (nmol/L)[†]	
Characteristic	n=344	<50 (n=98)	≥50 (n=246)
Age (yr.)	50.0 (5.1)	49.4 (4.8)	50.2 (5.2)
Height (cm)	164.0 (6.2)	164.3 (6.1)	163.9 (6.2)
Weight (kg)	73.7 (15.8)	78.8 (17.9)	71.7 (14.4)**
Body mass index (kg/m^2)	27.4 (5.8)	29.2 (6.5)	26.7 (5.3)**
Currently smoking n (%)	26 (7)	11 (11)	14 (6)*
Strenuous activity level	3.0 (1.4)	2.5 (1.4)	3.2 (1.3)**
Deseasonalized 25(OH)D (nmol/L)[†]	63.1 (22.1)	37.5 (8.6)	73.3 (17.0)**
Serum 25(OH)D level (nmol/L)	63.1 (22.8)	37.0 (10.5)	73.5 (17.4)**
Vitamin D supplement use n (%)	126 (36)	18 (18)	107 (44)**
Calcium supplement use n (%)	123 (35)	19 (20)	103 (42)**
Dietary calcium intake (mg/d)	1184 (494)	1215 (500)	1171 (493)
Menopausal status n (%)			
Post-menopause	86 (25)	22 (23)	63 (26)
Pre-menopause	134 (39)	47 (48)	84 (34)
Peri-menopause	102 (29)	20 (20)	82 (33)
Status unclear	26 (7)	9 (9)	17 (7)
Timed up and go test (seconds)	5.3 (0.7)	5.4 (0.7)	5.3 (0.7)
Step test (steps)	18.6 (4.7)	18.0 (2.4)	18.5 (2.7)
Functional reach test (cm)	41.2 (6.3)	41.1 (6.6)	41.3 (6.2)
Lateral reach test (cm)	18.7 (3.9)	19.1 (3.7)	18.6 (4.0)
Lower limbs muscle strength (kg)	75.8 (25.5)	74.9 (26.7)	76.1 (25.1)
Femoral neck BMD (g/cm^2)	0.814 (0.125)	0.825 (0.142)	0.809 (0.117)
Lumbar spine BMD (g/cm^2)	1.035 (0.151)	1.064 (0.162)	1.023 (0.145)*

[†]adjusted for season, see text for details.
25(OH)D, 25-hydroxyvitamin D; BMD, areal bone mineral density;
Values are Mean (SD) unless otherwise stated;
*p<0.05, **p<0.001 compared to deseasonalized 25(OH)D <50 nmol/L group.

Unadjusted and adjusted LOWESS scatter plots showing exploratory views of non-linear associations of serum 25(OH)D with multiple musculoskeletal outcomes are given in Figure 6.1 and Figure 6.2, respectively, with cut-points from nonlinear least squares estimation indicated by vertical lines.

Table 6.2 gives the adjusted and unadjusted cut-points with their 95% confidence intervals. Cut-points were similar and statistically significant for most outcomes in adjusted and unadjusted analyses, except for LRT (33 (1-64) nmol/L unadjusted and 42 (-8, 93) nmol/L adjusted, respectively).

Table 6.2: Unadjusted and adjusted cut-points for associations between deseasonalized serum 25(OH)D level and multiple musculoskeletal outcomes

	Cut-points of 25(OH)D (nmol/L)	
	Unadjusted	Adjusted
Femoral neck BMD (g/cm^2)	**32 (19, 45)**	**31 (18, 43)** †
Lumbar spine BMD (g/cm^2)	**35 (17, 54)**	**31 (17, 45)** †
Timed up and go test (seconds)	**34 (28, 40)**	**30 (24, 36)** ‡
Step test (steps)	**36 (29, 43)**	**33 (24, 41)** ‡
Functional reach test (cm)	**27 (16, 38)**	**31 (18, 43)** ‡
Lateral reach test (cm)	**33 (1, 64)**	42 (-8, 93) ‡
Lower limb muscle strength (kg)	**31 (19, 44)**	**29 (8, 49)** ‡

Bold denotes statistical significance, p<0.05, 25(OH)D, 25-hydroxyvitamin D; BMD, areal bone mineral density;
†Adjusted outcomes and deseasonalized 25(OH)D level were used, adjusted for weight, height, menopausal status, strenuous physical activity, dietary calcium intake and currently smoking status.
‡Adjusted outcomes and deseasonalized 25(OH)D level were used, adjusted for age, weight, height, educational level, strenuous physical activity and currently smoking status.

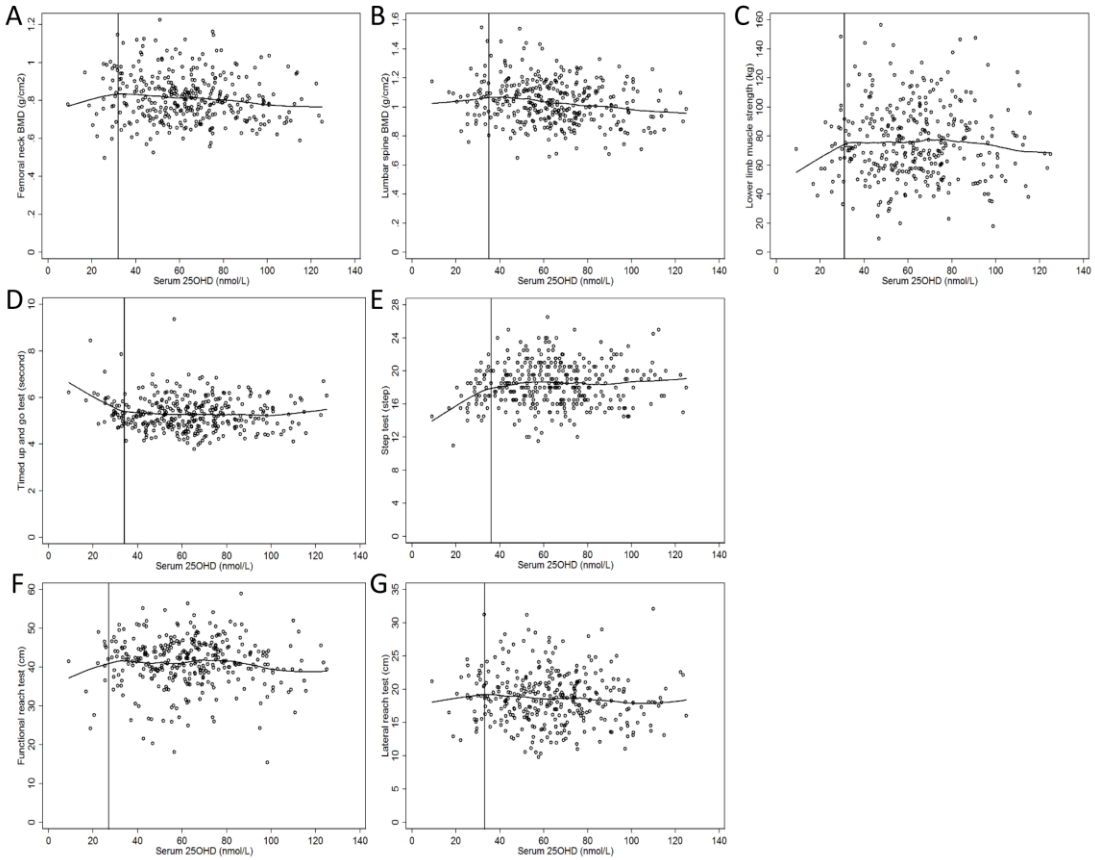

Figure 6.1: Unadjusted scatter plots and Locally weighted regression smoothing (LOWESS) curves for exploratory views of associations of serum 25(OH)D levels with multiple musculoskeletal outcomes, vertical lines indicate identified unadjusted cut-points (see Table 6.2) (raw data used).

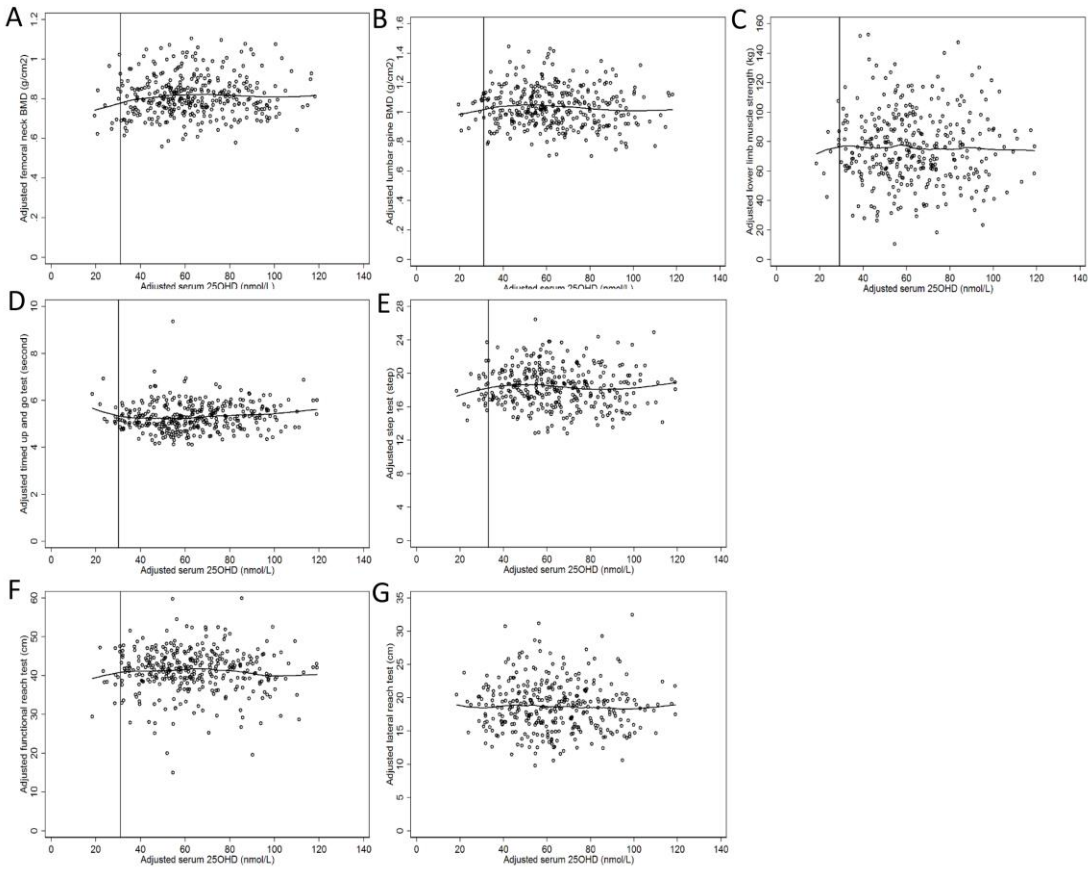

Figure 6.2: Adjusted scatter plots and locally weighted regression smoothing (LOWESS) curves for exploratory views of associations of serum 25(OH)D levels with multiple musculoskeletal outcomes, vertical lines indicate identified adjusted cut-points (see Table 2) (adjusted values for deseasonalized 25(OH)D level and outcomes used, see text for details).

Adjusted analyses for associations between 25(OH)D level and outcomes in participants with 25(OH)D level above and below the adjusted cut-points are given in **Table 6.3**. Below the cut-points, greater 25(OH)D levels were associated with increased FN and LS BMD (equivalent to an average improvement of 1% per nmol/L increase in 25(OH)D concentrations at each site) as well as improved TUG, ST FRT and LMS (equivalent to 1.8, 0.9, 1.1 and 4.3% improvements per nmol/L increase in 25(OH)D respectively). Above the cut-points, there were no beneficial associations and the only statistically significant association was a deleterious one, being a small increase in TUG.

Table 6.3: Associations between serum 25(OH)D level and multiple musculoskeletal outcomes[#] below and above adjusted cut-points of 25(OH)D level

	Cut-points	Below cut-point		Above cut-point	
		n	β (95% CI)	n	β (95% CI)
Femoral neck BMD (g/cm^2)[†]	31	10	0.008 (-0.001, 0.017)	333	0.0002 (-0.0003, 0.0008)
Lumbar spine BMD (g/cm^2)[†]	31	11	**0.010 (0.001, 0.018)**	332	-0.0005 (-0.0012, 0.0002)
Timed up and go test (seconds)[‡]	30	9	**-0.10 (-0.16, -0.04)**	330	**0.004 (0.001, 0.007)**
Step test (steps)[‡]	33	22	**0.16 (0.02, 0.31)**	317	-0.01 (-0.02, 0.003)
Functional reach test (cm)[‡]	31	10	0.44 (-0.21, 1.09)	329	-0.01 (-0.04, 0.02)
Lower limb muscle strength (kg)[‡]	29	5	**2.64 (0.74, 4.55)**	334	-0.04 (-0.17, 0.09)

Bold denotes statistically significant association within subgroup, p<0.05, 25(OH)D, 25-hydroxyvitamin D; BMD, areal bone mineral density

[#]Lateral reach test was not tested as there was not a significant adjusted cut-points (see table 2).

[†]Adjusted outcomes and deseasonalized 25(OH)D level were used, adjusted for weight, height, menopausal status, strenuous physical activity, dietary calcium intake and currently smoking status.

[‡]Adjusted outcomes and deseasonalized 25(OH)D level were used, adjusted for age, weight, height, educational level, strenuous physical activity and currently smoking status.

6.4 Discussion

To our knowledge, this is the first study to assess the optimal level of serum 25(OH)D for musculoskeletal health using multiple clinically important endpoints and the first examining associations with LMS and balance in a population-based sample of middle-aged women. Cut-points for associations between serum 25(OH)D level and the majority of outcomes were identified ranging from 29 to 33 nmol/L. Below these, greater 25(OH)D level is associated with increased FN and LS BMD, LMS and better performance on balance tests (an average improvement of 0.9% to 4.3% per nmol/L increase in 25(OH)D concentrations), while above them no beneficial associations were observed, suggesting these are minimum levels required for optimal musculoskeletal health.

Previous estimates of the optimal serum 25(OH)D level have been inconsistent probably due to methodological differences, for example differences in endpoints, study design and population, statistical methods and serum 25(OH)D assay methods. Accordingly, the choice of optimal 25(OH)D level for skeletal health remains controversial, with a range from 50 to 100 nmol/L supported by some but not all experts, though there is agreement that a level of less than 50 nmol/L is suboptimal for skeletal health[36]. The cut-points we identified should not be interpreted as values at which a sharp transition in slopes occurs, but rather a region of transition between strong and weak association as seen in the figures and as indicated by the wide 95% confidence intervals (CI) of the cut-points. The lower 95% CIs were less than 25 nmol/L for all outcomes and the upper 95% CIs ranging from 36 to 49 nmol/L.

Therefore, even though the cut-points of 29 to 33 nmol/L we identified are somewhat lower for most outcomes than the currently accepted cut-off of 50 nmol/L, this higher level may still be warranted for optimal musculoskeletal health.

The 1% greater BMD per 1 nmol/L higher serum 25(OH)D is a large effect size. In elderly women, it has been estimated that for each 5% loss in FN BMD there is a 40% and 90% increase in all fractures and hip fracture risk, respectively[37]. This may also apply in younger populations because BMD tracks throughout lifetime[38,39], i.e., people with lower BMD during midlife remain on a trajectory for having lower BMD than others into old age. If raising serum 25(OH)D in deficient women by 5 nmol/L could increase BMD by 5%, this would be a major and clinically important effect but ideally a randomised controlled trial is required to confirm whether this can be obtained. Such a trial of correcting vitamin D deficiency in middle-aged women with bone density outcomes should be a high research priority, though this would have to be carefully designed to avoid ethical issues with implementation.

The associations between 25(OH)D and other outcomes may also be clinically relevant. For example, in the case of muscle strength, A 25-yr prospective study of initially healthy middle-aged men (45-68 years old) showed that compared to those in the highest tertile of baseline grip strength, those in the lowest and middle tertiles were at greater risk of developing functional limitations and disabilities in old age (ORs ranging from 1.07 to 2.80)[40]. Similar long-term data in women are lacking. Interpreting effect sizes of balance tests is challenging in our setting because of a lack of studies. However, balance tests have been shown to accurately predict falls risk in older adults[26,28]. In addition, Zhu et al. showed that TUG test was a risk factor for

incident nonvertebral fracture in elderly women (hazard ratio = 1.54 (95% CI: 1.15-2.07) for <10.2 vs. >10.2 seconds), independent of BMD and other risk factors[41]. However, it should be noted that direct evidence that deficits in balance in middle-age have effects in older adult life is lacking. Given the lengthy period of follow-up required to assess such associations, this is likely to remain problematic.

Previous studies in this age group are limited. A previous large cross-sectional study by Bischoff-Ferrari et al. did not identify a cut-point for the relationship between serum 25(OH)D levels and hip BMD [42]. Higher serum 25(OH)D levels were associated with greater BMD in the hip throughout a reference range of 22.5 to 94 nmol/L, though most benefit was seen with a 25(OH)D level below ~50 nmol/L in younger women (aged 20 to 49 years) [42]. This is broadly consistent with our findings, though in our study there was an identifiable cut-point of 31 nmol/L and benefit of greater 25(OH)D concentrations on FN and LS BMD only existed in those with 25(OH)D level below this. One potential explanation for the discrepancy is the relatively higher calcium intake of 1186 mg/day in our study compared to 881 mg/d in the study by Bischoff-Ferrari et al.[42]. In elderly women with low calcium intake (< 800 mg/day) a higher serum 25(OH)D concentration (up to 120 nmol/L) is needed to keep PTH within the normal range[43]. Also, compared to the nonlinear least-squares estimation utilized in our study, the LOWESS used by Bischoff-Ferrari et al.[42] is an exploratory approach, which does not allow for an accurate determination of the cut-point.

In addition to the effect of vitamin D on calcium homeostasis and BMD, its protective effect on fractures may be mediated by improving lower-extremity function, thus

reducing falls risk[44]. Significant cut-points were identified for associations between serum 25(OH)D, LMS and most balance tests in our study, ranging from 29 to 33 nmol/L. Similar results were reported in a cross-sectional study in US older adults (aged ≥60 years), indicating that lower-extremity function (i.e. sit-to-stand test and 8-foot walk test) increased continuously with greater serum 25(OH)D level throughout a reference range from 22.5 to 94 nmol/L, with most of the improvement occurred in 25(OH)D level below around 40 nmol/L[44]. This is important because balance begins attenuating in midlife[6,7], and it has been suggested that the prevention of functional limitations in older age should begin in midlife[8]. Thus the potential for correcting vitamin D deficiency and maintaining ongoing adequate levels to improve muscle strength and balance in middle-aged women also appears worthy of exploration by carefully and ethically designed randomised controlled trials.

Surprisingly, there was an association with poor performance on TUG above the cut-point of 30 nmol/L, though the effect size was small. The reasons for this are unclear. One potential explanation is that high 25(OH)D levels may be detrimental for lower extremity function - a similar study in older adults found that participants at the highest 25(OH)D concentrations (>120 nmol/L) had impaired performance on sit-to-stand test[44]. Similarly, a RCT of an annual dose of 500000 IU of vitamin D3 in women aged over 70 years resulted in increased rates of falls and fractures in the vitamin D group, with a greater decline in muscle strength being observed in those with the greatest fluctuations in serum 25(OH)D levels[45]; however, the mechanism for getting very high serum 25(OH)D levels in these two studies might be different, supplement of a single bolus versus sunlight.

Our study has several limitations. One is the cross-sectional design, which means that causal associations between vitamin D and BMD, muscle strength and balance cannot be demonstrated. RCTs have shown that vitamin D supplementation improved serum 25(OH)D concentrations and decreased serum PTH levels in premenopausal women[46-48] but longitudinal and RCT data in younger women are otherwise lacking. As mentioned, well-designed RCTs are needed to directly confirm a causal relationship and determine the magnitude of any effect of improving vitamin D levels in women with sub-optimal levels[49-51]. The prevalence of low serum 25(OH)D was relatively low so there were relatively few women with 25(OH)D below the identified cut-points. This lowered the precision of estimating cut-points and associations below those cut-points, particularly for the LMS. Although the original study[24] had a population-based design, the participants were exposed to an osteoporosis behavioural intervention and there was a dropout rate of 26% by the end of final follow-up. There were some differences in sociodemographic characteristics and smoking behaviour between women retained in the study and those lost to follow-up but the wide spread of education levels at baseline and employment rate at 12 years approximates the overall population figures for these socioeconomic factors and adjustment for potential confounders was performed so our findings are still likely to apply to healthy middle-aged women from a range of sociodemographic backgrounds.

In conclusion, in these middle-aged Australian women cut-points for associations between serum 25(OH)D level and the majority of outcomes were observed, below which greater 25(OH)D level is associated with increased BMD and LMS as well as better performance on balance tests, while above which, there are no such associations.

A level of 25(OH)D of at least 29 to 33 nmol/L appears required for optimal musculoskeletal health in this population, but the current cut-off of 50 nmol/L may be warranted. Longitudinal studies are required to further confirm these findings and randomized controlled trials are necessary to accurately assess the effects of correcting vitamin D deficiency on musculoskeletal health in this age group.

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Chapter 7: Moderate-to-vigorous physical activity but not sedentary time is associated with musculoskeletal health outcomes in a cohort of Australian middle-aged women

7.1 Introduction

Bone mineral density (BMD), muscle strength and balance are all important aspects of musculoskeletal health. Low BMD is a major risk factor for fractures[1] and age-related loss of muscle strength is associated with decreased balance and functional limitations in older people[2,3]. Consequently, impaired balance and mobility increases risk of falls[4], conferring a high risk of fractures[5]. Declines in muscle mass, strength[6], BMD and bone strength[7,8] and balance start around 45-55 years of age[9,10], suggesting that prevention of functional limitation in older age should begin in early midlife[11], and in order to do so, ways to prevent age-related decline in BMD, muscle strength and balance need to be identified.

The amount of physical activity recommended for adults aged 18-64 years is 150 to 300 minutes of moderate-intensity aerobic physical activity or 75 to 150 minutes of vigorous-intensity aerobic physical activity weekly, or an equivalent combination[12]. However, few people achieve this, with one in five adults being physically inactive worldwide[13]. It is uncertain whether these recommendations are appropriate for musculoskeletal health in younger adults as few studies have collected data on both duration and intensity of physical activity using objective measures in younger adults, and their results are inconclusive[14-16]. Time spent in sedentary and sedentary behaviours is negatively associated with musculoskeletal health outcomes in older adults[17-19], but their effects in younger adults remain uncertain. It is also unclear

whether sedentary time and MVPA exert independent effects on musculoskeletal outcomes. The relative impact of sedentary behaviour compared to physical activity on musculoskeletal outcomes is important information for developing physical activity interventions and public health guidelines for musculoskeletal health.

Therefore, the aims of this study were to (1) describe associations of objectively-measured total physical activity, time spent at different intensities of physical activity and sedentary time with BMD, lower limb muscle strength (LMS) and balance measures in middle-aged women, and (2) examine whether any associations with MVPA are independent of sedentary time, and vice versa.

7.2 Materials and Methods

7.2.1 Participants

Participants were from a 10-yr follow-up of a previously reported two-year randomized controlled trial conducted in 2000 in Southern Tasmania, Australia. Details of the original trial have been reported elsewhere[20]. Briefly, women aged 25-44 years were randomly selected from the 2000 Tasmanian Electoral Roll. Women were excluded if they had previous measurement of BMD, thyroid disease, renal failure, malignancy, or rheumatoid arthritis, a history of hysterectomy, were taking hormone replacement therapies, pregnant or planning pregnancy within two years of study entry, or lactating. At baseline, 470 women were randomly assigned to one of two osteoporosis educational interventions: group education using the Osteoporosis Prevention and Self-management course (OPSMC) or an information leaflet. Participants had their BMD measured at the spine and hip at baseline, two years and

12. At baseline, those with a mean spine and hip T-score < 0 were informed that they were at a higher risk in later life whereas those with a mean T-score of zero or greater were informed that they were not at higher risk. The present study is a cross-sectional analysis of data obtained an additional 10-years after the original two-year trial was completed, comprising 347 women (aged 36-57 years). Participants were included in this analysis if they had at least one of the outcome measures performed and had at least five valid days of physical activity recorded (see below). Ethics approval was obtained from the Royal Hobart Hospital Ethics Committee, and all participants gave written informed consent.

7.2.2 Measurements

Measurement of physical activity and sedentary time

Accelerometers (ActiGraph GTlM) were used to measure ambulatory physical activity for seven consecutive days. Accelerometer counts were recorded in 60-second epochs. Participants were included in the analysis if they wore the accelerometer for at least 10 hours per day for five days in the week. Participants recorded start and finish times each day in a diary, as well as the duration and reason for any time where they took the accelerometer off and circumstances potentially affecting accelerometer readings (i.e. driving on uneven ground). Total physical activity was expressed as total accelerometer counts divided by total monitoring time per day (counts/minutes of wear time, CPM). A cut-off of < 150 CPM was used to define time spent sedentary[21,22]. Cut-offs for light physical activity and MVPA were 151-1748 and ≥ 1749 CPM, respectively[23]. Total sedentary time and time spent in light physical

activity and MVPA was divided by the number of valid days of accelerometer wear to produce an average time spent per day at each intensity.

BMD

BMD was measured at the lumbar spine (LS) and femoral neck (FN) by dual-energy X-ray absorptiometry (DXA) using fan beam setting on a Hologic Delphi bone densitometer (Hologic QDR2000, Waltham, MA), calibrated daily with coefficient of variation (CV) 1%.

Lower limb muscle strength (LMS)

LMS was measured to the nearest kilogram using a dynamometer (TTM Muscular Meter, Tokyo, Japan)[24]. This examines isometric strength, predominantly of the quadriceps and hip extensors. The examiner demonstrated the correct technique to the participant before testing. Participants stood on the back of the dynamometer platform, with back straight against a wall and knees flexed to an angle of 115 °. They lifted a bar connected to the dynamometer by a chain using maximum force using their legs, with the back and neck straight. The mean of two readings was calculated for analysis. The intraclass correlation coefficient for LMS was 0.94 (95%CI, 0.92, 0.95) in this study (from two-way random-effects model[25]).

Balance measurements

Balance was assessed using 4 clinical balance tests - the timed up and go test (TUG), the step test (ST), the functional reach test (FRT) and the lateral reach test (LRT),

with details described elsewhere previously[26]. For each test, an average value of all measures taken was calculated for use in analyses.

For the TUG participants sat in a normal armchair (45 cm high) with their back against the chair. The length of time required to stand up, walk a distance of 3 m, turn around, walk back and sit down again with their back against the chair was recorded. Two measures were performed.

For the ST, participants stood on one leg 5 cm from an 8.5-cm-high block positioned against a wall and placed the whole foot of the other leg onto the block and returned it to the floor repeatedly as fast as possible for 15 seconds, with the number of steps recorded. Both legs were tested.

For the FRT, participants stood with their feet behind a line perpendicular and adjacent to a wall. The arm closest to the wall was raised to shoulder height and the position of the knuckle of the middle finger marked. Keeping the feet flat, participants leaned forward as far as possible and the position of the knuckle was recorded at the point of furthest reach. FRT was the difference between the two measures. Each side was measured three times.

For the LRT, participants stood with their backs near but not touching a wall with the heels 10 cm apart. Participants raised both arms to shoulder height and maintained equal weight bearing while the position of the third finger's tip on the side being measured was marked on the wall. Participants then lowered the arm not being measured and reached sideways as far as possible with the arm being measured and

the position of furthest reach marked. The difference between the two marks was calculated. Each side was measured three times.

Serum 25(OH)D levels

Venous blood samples were taken. Serum 25-hydroxy vitamin D (25(OH)D) level was assayed using liquid chromatography (LC)-tandem mass spectrometry ((LC-MS/MS). The assay measures $25(OH)D_2$ and $25(OH)D_3$ separately with a CV 3-6%, using an internal standard.

Dietary intake

We estimated habitual dietary intake by using a food frequency questionnaire (Anti-Cancer Council of Victoria), which has been validated against 7-day food diaries with energy-adjusted correlation coefficients for nutrient intakes ranging from 0.28 for vitamin A to 0.78 for carbohydrate (22). Intakes of calcium, energy, fat, protein, carbohydrate, cholesterol, iron, magnesium, phosphorus, sodium, vitamin C, vitamin E, and zinc were calculated using Nutrient tables for use in Australia (NUTTAB) 2010.

Anthropometry and other factors

Height was measured by stadiometer (The Leicester height measure, Invicta Plastics Ltd, Oadby, England), weight by a single set of calibrated scales (Heine, Dover NH USA). Body mass index (BMI) was calculated (weight (kg)/height $(m)^2$). Other items were assessed using questionnaires: education level, employment status of main financial provider in the household, smoking history (current/former/never), marital

status, number of children, breastfeeding history, menopausal status, personal history of fracture (all types including high trauma), and family history of osteoporosis and/or fracture.

7.2.3 Statistical analysis

Mean (SD) and median (interquartile range) were used to describe continuous variables as appropriate. Number (%) was used to describe categorical variables. Univariable and multivariable linear regression models were used to describe associations between total physical activity (counts per minute of wear time (CPM)), time spent sedentary (minutes/day) and in light physical activity (minutes/day) and in MVPA (minutes/day) with each of the outcomes, adjusting for potential confounders.

To explore independent associations between sedentary time and time spent in light physical activity and MVPA with each outcome, we further adjusted for MVPA time when sedentary time was the main exposure of interest and vice versa. When light physical activity time was the exposure of interest, we adjusted for MVPA time but not sedentary time due to substantial collinearity between sedentary time and time spent in light physical activity, as indicated in other literature[27].

Sensitivity analyses were performed by fitting models using different cut-points taken from the literature in younger adults for different physical activity intensities[21,28-30]. These were: sedentary time (< 250 CPM); light physical activity (150-1951, 150-2191 CPM when 150 CPM was used for sedentary time, 250-1748, 250-1951, 250-2191 CPM when 250 was used for sedentary time) and MVPA (> 1951 or 2191 CPM as appropriate according to the cut-point for light physical activity).

All analyses were performed in Stata version 12 (Stata Corporation, Texas, USA).

7.3 Results

Of the 347 women, 37 had fewer than five valid days of accelerometer results and one did not have any outcome measured, thus 309 women were included in this analysis. Characteristics of participants are shown in Table 7.1. The mean of total physical activity was 376 CPM. The median for total accelerometer wear time, sedentary time, and time spent in MVPA were 851, 535 and 37 minutes/day, respectively (Table 7.1).

Table 7.1: Characteristics of participants (n=309)

Characteristic	Mean (SD)[a]
Age (yr.)	50 (5)
Height (cm)	164.2 (6.1)
Weight (kg)	73.1 (15.3)
Body mass index (kg/m^2)	27.1 (5.5)
Serum 25(OH)D level (nmol/L)	63.7 (23.2)
Dietary calcium intake (mg/d)	1184.7 (492.0)
Menopausal status, n (%)	
Pre-menopause	118 (37.9)
Peri-menopause	89 (28.8)
Post-menopause	82 (26.5)
Status unclear	21 (6.8)
History of fracture, n (%)	125 (40.5)
Currently smoking, n (%)	22 (7)
Timed up and go test (seconds)	5.292 (0.696)
Step test (steps)	18.37 (2.52)
Functional reach test (cm)	41.38 (6.20)
Lateral reach test (cm)	18.66 (3.88)
Lower limbs muscle strength (kg)	75.42 (25.27)
Femoral neck BMD (g/cm^2)	0.810 (0.124)
Lumbar spine BMD (g/cm^2)	1.029 (0.150)
Total physical activity (counts/min)	376 (151)
Time (minutes) (median, IQR)	
Total accelerometer wearing	851(810-893)
Spent sedentary	535 (474-596)
Spent in light physical activity	267 (229-309)
Spent in MVPA	37 (25-58)

[a]Mean (SD) unless otherwise stated.
25(OH)D, 25-hydroxyvitamin D; BMD, bone mineral density; MVPA, moderate-to-vigorous physical activity; IQR, interquartile range.
Sedentary, < 150 counts/min (CPM); light physical activity, 150-1749 CPM; MVPA, ≥1749 CPM.

After adjustment for confounders (Table 7.2), total physical activity (per 100-CPM) was positively associated with FN BMD (β = 0.011 g/cm^2, 95% confidence interval (CI): 0.003, 0.019) and LMS (β = 2.13 kg, 95% CI: 0.21, 4.06), while negatively but beneficially associated with TUG (β = -0.080 seconds, 95% CI: -0.129, -0.030). These effects equate to approximately 1.4%, 2.8%, and 1.5% difference from the average values of the study sample for FN BMD, LMS, and TUG, respectively. The direction of effect was also positive for the ST (β = 0.18 steps, 95% CI: -0.01, 0.36). There were no associations between total physical activity and LS BMD, FRT and LRT.

Table 7.2: The association of total physical activity (counts/min of wear time, CPM) with BMD, lower limb muscle strength and balance (n=309)

	Unadjusted	Adjusted[b]
	β[a] (95% CI)	β[a] (95% CI)
Femoral neck BMD (g/cm^2)	0.006 (-0.003, 0.015)	**0.011** (**0.003**, **0.019**)
Lumbar spine BMD (g/cm^2)	-0.002 (-0.013, 0.009)	0.004 (-0.007, 0.014)
Lower limbs muscle strength (kg)	1.77 (-0.10, 3.63)	**2.13** (**0.21**, **4.06**)
Timed up and go test (seconds)	**-0.116 (-0.166**, **-0.066**)	**-0.080** (**-0.129**, **-0.030**)
Step test (steps)	**0.27 (0.09**, **0.46**)	0.18 (-0.01, 0.36)
Functional reach test (cm)	0.40 (-0.06, 0.86)	0.40 (-0.05, 0.86)
Lateral reach test (cm)	-0.07 (-0.36, 0.22)	-0.002 (-0.30, 0.30)

BMD, bone mineral density.
[a]Coefficients represent the change in the outcome for a 100-CPM change in total physical activity. Bold denotes statistical significance, p<0.05.
[b]Adjusted for age, weight, height and menopausal status, calcium intake, serum 25-hydroxyvitamin D levels and history of fracture.

After adjustment for potential confounders (Table 7.3 Model 2), MVPA (per 10-minutes) was beneficially associated with FN BMD (β=0.0050 g/cm^2, 95% CI: 0.0007, 0.0094), LMS (β = 1.48 kg, 95% CI: 0.45, 2.52), ST (β = 0.12 steps, 95% CI: 0.02, 0.23), and TUG (β = -0.043 seconds, 95% CI: -0.070, -0.016) (Table 7.3 Model 2); this equates to approximately 0.6%, 2.0%, 0.7 % and 0.8% difference from the average values of the study sample for FN BMD, LMS, ST and TUG, respectively. Time spent in light physical activity was not associated with any outcome (Table 7.3). An increase in sedentary time of 60 minutes/day was detrimentally associated with

TUG (β = 0.075 seconds, 95% CI: 0.013, 0.137), equating to around a 1.4% increase in TUG time. There were no statistically significant associations with any other outcomes.

After additionally adjusting for sedentary time, the association between MVPA and FN BMD was attenuated (β = 0.0041 g/cm^2, 95% CI: -0.0008, 0.0090). The association with TUG was also attenuated but remained (β = -0.035 seconds, 95% CI: -0.066, -0.005). In contrast, adjusting for sedentary time strengthened associations of MVPA with LMS (β = 1.63 kg, 95% CI: 0.45, 2.81) and ST (β = 0.15 steps, 95% CI: 0.04, 0.26) (Table 7.3 Model 3). The association between sedentary time and TUG did not persist after adjustment for MVPA (Table 7.3 Model 3).

In sensitivity analyses, using different cut-points for physical activity intensity levels did not affect the results (data not shown).

Table 7.3: Associations between sedentary time and time spent in light physical activity and in MVPA with musculoskeletal health outcomes (n=309)

	β (95% CI)[a]		
	Sedentary time	Light physical activity	MVPA
Unadjusted			
Femoral neck BMD (g/cm^2)	-0.0090 (-0.0186, 0.0007)	0.0009 (-0.0012, 0.0030)	0.0002 (-0.0032, 0.0067)
Lumbar spine BMD (g/cm^2)	-0.0007 (-0.0125, 0.0110)	-0.0005 (-0.0031, 0.0021)	-0.0018 (-0.0078, 0.0042)
Lower limb muscle strength (kg)	0.36 (-1.62, 2.33)	0.05 (-0.39, 0.49)	**1.15 (0.15, 2.16)**
Timed up and go test (seconds)	**0.077 (0.023, 0.130)**	-0.010 (-0.022, 0.002)	**-0.059 (-0.086, -0.031)**
Step test (steps)	-0.07 (-0.26, 0.13)	-0.02 (-0.06, 0.03)	**0.16 (0.06, 0.26)**
Functional reach test (cm)	-0.16 (-0.65, 0.32)	-0.002 (-0.11, 0.10)	0.17 (-0.07, 0.42)
Lateral reach test (cm)	0.27 (-0.03, 0.57)	0.01 (-0.06, 0.08)	-0.02 (-0.17, 0.14)
Adjusted[b]			
Femoral neck BMD (g/cm^2)	-0.0090 (-0.0189, 0.0010)	0.0010 (-0.0009, 0.0028)	**0.0050 (0.0007, 0.0094)**
Lumbar spine BMD (g/cm^2)	-0.0005 (-0.0134, 0.0123)	-0.0002 (-0.0026, 0.0022)	0.0018 (-0.0038, 0.0074)
Lower limb muscle strength (kg)	-1.06 (-3.46, 1.34)	-0.05 (-0.50, 0.40)	**1.48 (0.45, 2.52)**
Timed up and go test (seconds)	**0.075 (0.013, 0.137)**	-0.008 (-0.020, 0.004)	**-0.043 (-0.070, -0.016)**
Step test (steps)	-0.02 (-0.25, 0.22)	-0.02 (-0.06, 0.02)	**0.12 (0.02, 0.23)**
Functional reach test (cm)	-0.17 (-0.73, 0.40)	0.003 (-0.10, 0.11)	0.18 (-0.07, 0.43)
Lateral reach test (cm)	-0.03 (-0.39, 0.34)	-0.002 (-0.07, 0.07)	0.04 (-0.12, 0.20)
Adjusted[c]			
Femoral neck BMD (g/cm^2)	-0.0046 (-0.0158, 0.0066)	0.0005 (-0.0013, 0.0023)	0.0041 (-0.0008, 0.0090)
Lumbar spine BMD (g/cm^2)	0.0018 (-0.0127, 0.0164)	-0.0005 (-0.0028, 0.0018)	0.0022 (-0.0042, 0.0086)
Lower limb muscle strength (kg)	0.71 (-1.98, 3.41)	0.0001 (-0.44, 0.44)	**1.63 (0.45, 2.81)**
Timed up and go test (seconds)	0.036 (-0.033, 0.106)	-0.006 (-0.017, 0.005)	**-0.035 (-0.066, -0.005)**
Step test (steps)	0.15 (-0.11, 0.41)	-0.02 (-0.06, 0.02)	**0.15 (0.04, 0.26)**
Functional reach test (cm)	0.03 (-0.61, 0.66)	-0.01 (-0.11, 0.09)	0.18 (-0.10, 0.46)
Lateral reach test (cm)	0.02 (-0.39, 0.44)	0.02 (-0.05, 0.09)	0.05 (-0.13, 0.23)

BMD, bone mineral density; MVPA, moderate-to-vigorous physical activity.

[a]Coefficients represent the change in the outcome for a 60-minutes change in time spent sedentary and a 10-minutes change in time spent in light physical activity and MVPA. Bold denotes statistical significance, $p<0.05$.

[b]Adjusted for age, weight, height, menopausal status, calcium intake, serum 25-hydroxyvitamin D levels, history of fracture and accelerometer wear minutes.

[c]Sedentary time and light physical activity were additionally adjusted for MVPA and MVPA was additionally adjusted for sedentary time.

7.4 Discussion

This is the first study, of which we are aware, to describe the independent associations between objectively-measured physical activity and time spent sedentary with musculoskeletal health outcomes in a population-based sample of middle-aged women. Each additional 100-counts/min of total physical activity was associated with 1.4% greater FN BMD, 2.8% greater LMS, 1.0% more steps in ST and 1.5% shorter TUG. Intensity of physical activity was also important: MVPA was beneficially associated with FN BMD, LMS, TUG and ST but light physical activity and sedentary time were not independently associated. Effects of MVPA on LMS, TUG, ST but not FN BMD were independent of sedentary time, but not vice versa, suggesting that increasing time spent in MVPA may be more important than decreasing sedentary time for musculoskeletal health in middle-aged women, and sedentary time may not be influential when adequate MVPA is achieved.

The effects observed are of sufficient magnitude to be clinically meaningful. For example, there is an average loss of 1.0% and 1.4% per year for total hip BMD in the late perimenopausal period [31], and in our study, a relatively modest 10-minute increase in MVPA was associated with FN BMD improvement equivalent to approximately 0.6%. Similarly, the effect sizes for LMS (2.0%), ST (0.7%) and TUG (0.8%) compare favourably to annualized decline of 2.2% and 2.5% for grip strength, 0.3% and 1.6% for balance (the standard Romberg test), and 0.19% and 0.25%% for gait velocity observed in women aged 50 and 60 years at baseline, respectively[32].

Existing data on associations between objectively-measured physical activity and sedentary time and BMD are sparse in middle-aged women[14-16]. In line with our

study, two small studies found that activities of higher intensity producing higher loading were significantly associated with broadband ultrasound attenuation at the heel[14] and change in BMD at the proximal femur[15] in middle-aged women. However, a 6-yr longitudinal study in 244 women aged 35-45 years reported that total physical activity was associated with change in hip BMD, but intensity was not[16], but only small numbers of women were classified as having moderate and vigorous intensity (27 and 17 participants respectively) limiting the study's power. Chastin et al. reported a negative association between sedentary behaviour and total femur BMD in women aged 23 to 90+ years[27] but did not report specifically on effects in younger women. The association was independent of MVPA. Neither this study nor ours observed any association between sedentary time and LS BMD. Overall, the importance of the impact of sedentary time on bone density in middle-aged women remains unclear.

Data on other musculoskeletal outcomes are sparse but generally consistent with our results. Willoughby et al. showed that greater MVPA was associated with greater peak torque of knee flexors and better postural stability, independent of time spent sedentary[33] but that the deleterious effects of sedentary time were not independent of MVPA. Together with our findings, this highlights the potential importance of increasing MVPA for maintaining muscle strength and balance, regardless of the amount of time spent sedentary. Chahal et al. found that both MVPA and light physical activity were associated with knee extension torque in middle-aged women (n = 34; mean (SD) age = 49.8 (7.5) years)[14], but as light physical activity included fast walking which is often considered as MVPA[34], their findings are essentially consistent with ours.

It is not surprising that the effects of physical activity on musculoskeletal outcomes, particularly bone density would be related to intensity. Physical activity produces dynamic mechanical loads that influence bones through ground reaction forces and by the contractile activity of muscles[35], and it is suggested that a threshold of mechanical strain magnitude should be reached before osteogenic stimulus is initiated[36]. There is strong evidence from clinical controlled trials indicating that exercises including high impact loading (e.g., resistance training) are most beneficial for improving BMD in premenopausal women[37,38]. Similar evidence for LMS and balance are lacking, being restricted to a pre-post intervention study showing that improved LMS and balance from strength training using a combination of maximal and explosive strength training protocols in middle-aged women[39]. However, RCTs of different intensities of ambulatory physical activity for bone density, LMS and balance are lacking and are needed to compare the importance of different intensities of ambulatory physical activity for improving bone density, muscle strength and balance in younger women.

Our study has some limitations. First, causality cannot be inferred due to the cross-sectional design - we cannot exclude the possibility that those who have poorer BMD, muscle strength and balance are less likely to be physically active. However, intervention studies have shown that high-impact activity with high-magnitude resistance training are effective for improving BMD in premenopausal and postmenopausal women[37,40], suggesting that some degree of causation is likely. Nonetheless, RCTs of different intensities of ambulatory physical activity for bone density, LMS and balance are needed to confirm the causality and determine the optimal physical activity advice to give younger women for improving these musculoskeletal outcomes. Second, the cut-offs used for sedentary time, and physical activity intensities could influence the results; however, in sensitivity analyses using a range of published cut-offs the results remained largely unchanged, suggesting this is

not a significant issue. Finally, while our original study used population-based sampling[41], the generalizability of this analysis might be reduced due to the fact that it is a cross-sectional analysis of data from the 74% of the cohort retained after 12 years. However, women who completed 12-yr follow-up had similar characteristics to those who were lost to follow-up, other than being slightly (2 years) older (see chapter 5). Moreover, while there were some differences in smoking status and sociodemographic variables[42], the wide spread of education levels at baseline and employment rate at 12 years approximates the overall population figures for these socioeconomic factors and adjustment for potential confounders was performed. Therefore, our findings are still likely to apply to healthy middle-aged women across a range of sociodemographic characteristics.

In summary, our study showed that in middle-aged women, greater total physical activity (CPM) was associated with better musculoskeletal health. Moderate to vigorous physical activity appears more important than light or sedentary activity for many musculoskeletal outcomes in younger women. These findings are important for developing interventions to improve habitual physical activity that are targeted at improving musculoskeletal health among women in midlife when an accelerated process of decline in BMD, muscle strength and balance begins.

7.5 References

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Chapter 8: Lower limb muscle strength is associated with poor balance in middle-aged women: linear and nonlinear analyses

8.1 Introduction

Falls are a major health issue among older adults. Approximately one in three people aged 65 years and over fall each year[1,2], with rates increasing with advancing age[3]. Falls result in substantial injury and mortality, accounting for 20-30% of moderate to severe injuries[4] and 40% of all injury-related deaths in the elderly[5].

Adequate balance and mobility are critical in maintaining independence in activities of daily living. Impaired balance and mobility increases risk of falls[6], with 4-39% of falls in people older than 65 years accounted for by gait/balance disorders [5]. Importantly, balance begins attenuating after 45-55 years of age[7,8], particularly in women, leading to suggestions that prevention of functional limitations in older age should begin in early midlife[9].

Age-related loss of muscle strength is an important contributor to decreased balance and functional limitations in older people[10,11] but has rarely been investigated in young[12] or middle-aged adults[13]. Although linear associations between muscle strength and physical performance (e.g. walking speed and balance) have been widely reported, results of the few studies examining nonlinear associations in older people[14-16] suggest there is a potential cut-point below which muscle strength is more strongly associated with physical performance, and above which there is only a weak or no relationship. Such cut-points are potentially clinically important as they could

be used to identify people at higher risk of developing balance problems and falls, and they may suggest a level of strength that interventions should aim to produce to improve physical function. However, it is currently unknown if such cut-points exist in middle-aged women.

Therefore, the aims of this study were to: 1) describe associations between lower limb muscle strength and balance measures in middle-aged women, and 2) determine whether there is evidence for thresholds where the associations change.

8.2 Materials and Methods

8.2.1 Study sample

The study sample for this cross-sectional analysis comprised 345 women (mean age of 49.9 years, 36.2 to 56.8 years of age) obtained at the end of the 10-year follow-up of a 2-year osteoporosis randomized controlled trial in Southern Tasmania, Australia, with details reported previously[17]. Briefly, women aged 25-44 years were randomly selected from the Tasmanian Electoral Roll in 2000. Women were recruited if they were free of the following: previous measurement of bone density, history of thyroid disease, renal failure, malignancy, rheumatoid arthritis, hysterectomy, hormone replacement therapies, pregnancy or planning pregnancy within 2 years of study entry, or lactating. At baseline, 470 women were randomly assigned to one of two osteoporosis educational interventions: group education using the Osteoporosis Prevention and Self-management course (OPSMC) or an information leaflet. Participants had their bone mineral density measured at the spine and hip at baseline, 2 and 12 years. At baseline, those with a mean spine and hip T-score <0 were informed that they were at a higher risk in later life whereas those with a mean T-

score of 0 or greater were informed that they were not at higher risk. Ethics approval was obtained from the Tasmania Health and Medical Human Research Ethics Committee and all participants gave written informed consent.

8.2.2 Measurements

Balance

We measured 4 clinical balance tests: the timed up and go test (TUG), the step test (ST), the functional reach test (FRT) and the lateral reach test (LRT). These assess balance performance from either a static or dynamic aspect, and are able to differentiate between fallers and non-fallers in older adults[18]. All have been validated in older women and have a high reliability, with normative values determined in women of the age in our study[18-20].

TUG[21] is a test of dynamic steady-state balance and gait. Participants sat in an armchair (45 cm high) with their back against the chair, then stood without using the arms, walked 3 m using a comfortable and safe walking speed, turned, walked back, and sat down. The average time of two trials was used for analysis.

The ST[22] measures speed of performing a dynamic stepping task. Participants stood 5 cm from an 8.5-cm-high block positioned against a wall and placed the whole foot of one leg onto the block and then returned it to the floor repeatedly as fast as possible for 15 seconds. The number of steps was recorded. Both sides were tested, and the mean number of steps for each side was calculated for analysis.

The FRT measures ability to reach forward with each arm from a bilateral stance position[19]. Participants stood with feet a comfortable distance apart behind a line

perpendicular and adjacent to a wall. The arm closest to the wall was raised to shoulder height and the position of the knuckle of the middle finger marked[19]. Participants leaned forward as far as possible and distance of the knuckle from the first mark is recorded. The mean of three trials on each side was calculated for analysis.

The LRT measures ability to reach to the side in bilateral stance[20]. Participants stood with their backs near but not touching a wall with the heels 10 cm apart. Participants raised both arms to shoulder height while the position of the third finger's tip on the side being measured was marked. Participants then lowered the arm not being measured and reached sideways as far as possible with the arm being measured. The position of furthest reach was marked and the difference between the two marks calculated. The mean of three trials on each side was calculated for analysis.

Lower limb muscle strength (LMS)

LMS was measured to the nearest kilogram using a dynamometer (TTM Muscular Meter, Tokyo, Japan)[23] to assess isometric strength, predominantly of the quadriceps and hip extensors. The examiner demonstrated the correct technique to the participant before testing. Participants stood on the back of the dynamometer platform, with back straight against a wall and knees flexed to an angle of 115 °. They held a bar, connected to the dynamometer by a chain, and lifted the bar using maximum force using their legs, with the back and neck straight. Two readings were made, and the mean calculated for analysis. The intraclass correlation coefficient for LMS was 0.94 (95%CI, 0.92, 0.95) in this study (from two-way random-effects model[24]).

Other measurements

Strenuous and light physical activity levels were measured by a validated questionnaire[25], which was modified for Tasmanian conditions and has been used previously in women of this age[26]. It asked how many days in the last 14 the participants reported performing at least 20 minutes of strenuous exercise and light exercise, measured in five categories (1 = 0 days, 2 = 1-2 days, 3 = 3-5 days, 4 = 6-8 days, 5 = 9 or more days). Participants were also asked to recall if they had regularly used *calcium and vitamin D supplements* during the last year, where regular use means taking supplements at least 5 times per week for more than 9 months of the year. The information of prescription medication was collected by asking participants to report all medication, prescribed by a doctor that they had taken in the last 2 weeks. *Anthropometric factors* included height measured by a stadiometer (The Leicester height measure, Invicta Plastics Ltd, Oadby, England), weight by a single set of calibrated scales (Heine, Dover NH USA) and body mass index (BMI) (calculated as weight/height^2 (kg/m^2)). A standardised questionnaire was used to collect smoking history (current/former/never), family history of osteoporosis and/or fracture, and previous fractures (yes/no and site), education level, employment status of main financial provider in the household, menopausal status, and marital status.

8.2.3 Statistical analyses

Linear associations between LMS and TUG, ST, FRT and LRT were estimated using univariable and multivariable linear regression, adjusting for confounding by age, weight, height, education level and strenuous physical activity. Locally weighted regression smoothing (LOWESS) was used to assess evidence for nonlinear associations between LMS and balance tests. Nonlinear least-squares estimation was then used to estimate the cut-points where the slope changes, and segmented

regression to estimate the slopes (beta coefficients) for participants with LMS below and above the identified cut-points. To adjust for potential confounding, adjusted balance test measures and LMS were used in LOWESS, nonlinear least-squares estimation and segmented regression. Adjusted values for each balance test were calculated by regressing each measured balance test on its confounding factors, and then adding the residuals to the mean of each measured balance test. Adjusted LMS was calculated by the same approach. We selected potential confounders by three steps: Step 1: we performed univariable regression for each potential confounder, and only those with $p<0.20$ were further considered in the next step. Step 2: age, weight and height were included as a compulsory covariate and all other variables identified in step 1 were included in the initial multivariable model. Step 3: besides age, adjustments for other covariates were made only if including a covariate changed the estimated coefficient of the exposure of interest (LMS) by more than 10%.

The goodness of fit of the regression models was evaluated, and sensitivity analyses performed by fitting models after excluding influential observations. All analyses were performed in Stata version 12 (Stata Corporation, Texas, USA). A two-tailed p value <0.05 was considered statistically significant.

8.3 Results

In the original study, a total of 470 women (64% response rate) aged 25-44 years were recruited at baseline. Three women withdrew after baseline assessments were performed but before bone density was assessed. Of the remaining 467 women, 347 (74%) were retained at 12 years and 345 women with full data for LMS and balance were analysed in the present study. Baseline characteristics of participants who did and did not complete the follow-up have been previously reported[27]. Briefly, women

lost to follow-up were younger, had lower levels of education, and were more likely to be current or past smokers, and less likely to be married or in a de facto relationship compared to those who were retained. Other anthropometric and demographic factors were comparable.

Participants' ages ranged from 36-57 years. Other characteristics are given in Table 8.1. Unadjusted linear associations between LMS and balance tests are shown in Figure 8.1. Greater LMS was associated with faster TUG (β=-0.009, 95%CI: -0.012, -0.006; second/kg), greater number of steps on the ST (β=0.032, 95%CI: 0.013, 0.052; step/kg), and further distance on the FRT (β=0.093, 95%CI: 0.068, 0.117; cm/kg) and LRT (β=0.036, 95%CI: 0.020, 0.052; cm/kg). These associations persisted after adjustment for potential confounders (Table 8.2).

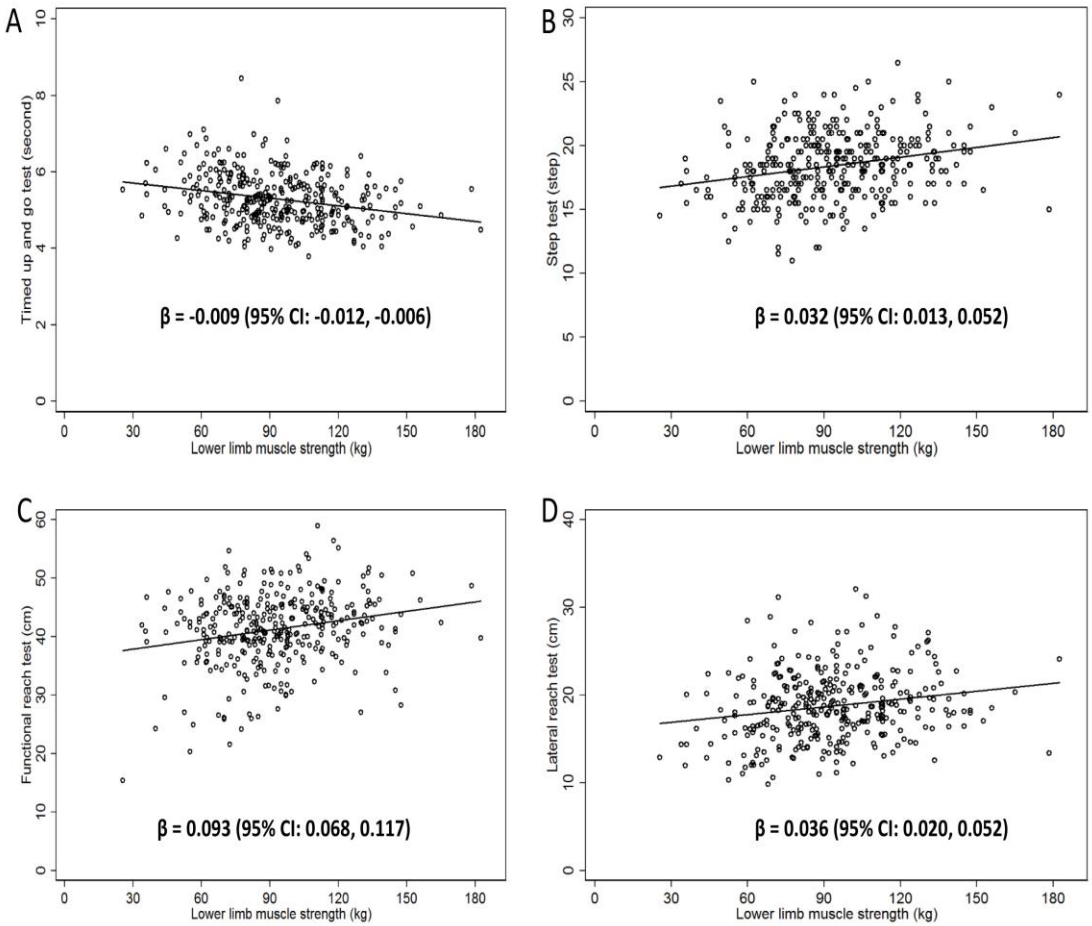

Figure 8.1: Scatter plots and linear regression lines for associations of lower limb muscle strength and balance. Beta coefficients and corresponding 95% confidence intervals from univariable linear regression are presented. Bold denotes statistically significant, p<0.001. Higher values of timed up and go test represent poorer performance whereas higher values of all the other tests represent better performance.

Table 8.1: Characteristics of study participants (n=345)

Characteristic	Value
Age (years)	49.9 (5.2)
Height (cm)	164.0 (6.1)
Weight (kg)	73.8 (15.8)
Body mass index (kg/m^2)	27.4 (5.8)
Strenuous activity level	3.0 (1.4)
Calcium supplement n (%)	123 (35)
Vitamin D supplement n (%)	126 (36)
Use of antihypertensive n (%)	55 (16)
Use of antidepressant n (%)	56 (16)
Use of hypnotic n (%)	4 (1)
Menopausal status n (%)	
Post-menopause	86 (25)
Pre-menopause	134 (38)
Status unclear	26 (7)
Perimenopausal	102 (29)
History of fracture n (%)	141 (41)
No. of fractures[a]	200
Upper limb n (%)	95 (48)
Lower limb n (%)	67 (33)
Others n (%)	38 (19)
Family history n (%)	
Osteoporosis	120 (35)
Fracture	241 (69)
Timed up and go test (seconds)	5.30 (0.71)
Step test (steps)	18.6 (4.7)
Functional reach test (cm)	41.2 (6.3)
Lateral reach test (cm)	18.7 (3.9)
Lower limb muscle strength (kg)	75.6 (25.4)

Values are Mean (SD) unless otherwise stated.
[a]including high trauma fractures.

Table 8.2: Linear regression for the association of lower limb muscle strength (LMS) with balance

	Lower limb muscle strength (kg)	
Balance measures	β (95%CI)[†]	β (95%CI)[‡]
Timed up and go test (seconds)	**-0.009 (-0.011, -0.006)**	**-0.008 (-0.010, -0.005)**
Step test (steps)	**0.027 (0.017, 0.037)**	**0.031 (0.011, 0.051)**
Functional reach test (cm)	**0.077 (0.052, 0.101)**	**0.071 (0.047, 0.096)**
Lateral reach test (cm)	**0.031 (0.014, 0.047)**	**0.028 (0.011, 0.044)**

Bold denotes statistical significance, p<0.05.
Higher values of timed up and go test represent poorer performance whereas higher values of all the other tests represent better performance.
[†]Adjusted for age, weight and height.
[‡]Further adjusted for education level and strenuous physical activity.

Figure 8.2, shows scatter plots with LOWESS curves for LMS and adjusted balance test data suggesting potentially nonlinear associations. Nonlinear least-squares estimation identified statistically significant cut-points for TUG [29 (95% CI: 24, 33) kg], ST [47 (28, 66) kg], FRT [50 (14, 85) kg] and LRT [33 (12, 54) kg] (Table 8.3).

Associations between LMS and balance measures in women with LMS above and below the identified cut-points are given in Table 8.3. There were significant associations between LMS and all balance measures in women with LMS both below and above the LMS cut-points except for LRT, where the association was only significant in women with LMS above the cut-point. However, the magnitude of the effects of LMS on balance tests were consistently greater in participants with LMS below the cut-points compared to those above.

Assessment of goodness of fit for the regression models revealed several potentially influential observations. In sensitivity analyses without the influential data points, the estimated associations for the linear regression models were similar to those from the full models (data not shown). However, the cut-points estimated in the nonlinear

analyses were no longer identifiable or statistically significant for all outcomes, other than ST (50 (95% CI: 32, 67) kg). Associations between LMS and ST were significant in women with LMS both below and above the LMS cut-point (0.081 (95% CI: 0.030, 0.133) vs. 0.014 (0.002, 0.026) respectively).

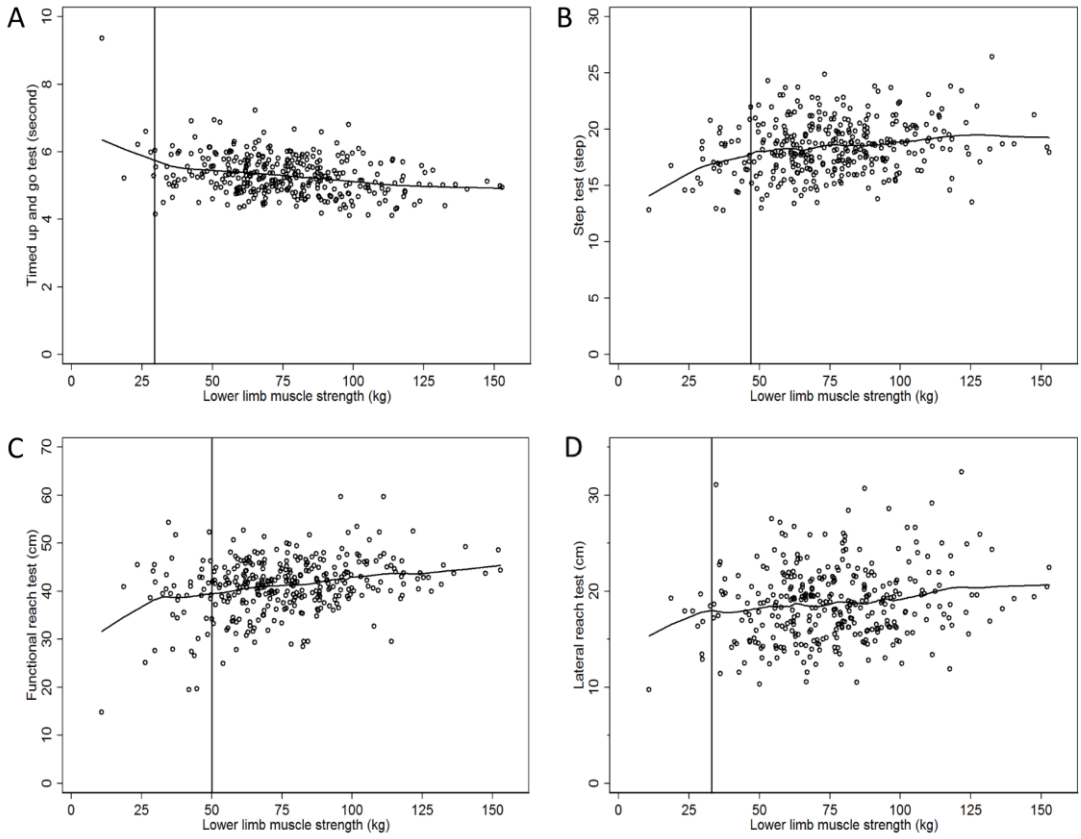

Figure 8.2: LOWESS scatter plots for adjusted lower limb muscle strength and balance tests, vertical lines indicate cut-points identified by nonlinear least-squares estimation (adjusted values used, see statistical section for details). Higher values of timed up and go test represent poorer performance whereas higher values of all the other tests represent better performance.

Table 8.3: Cut-points for associations between lower limb muscle strength (LMS) and balance, and associations in participants with LMS below or above the identified cut-points†

	Lower limb muscle strength (kg)				
	Cut-points	Below cut-point		Above cut-point	
Balance measures		n	β (95% CI)	n	β (95% CI)
Timed up and go test (seconds)	**29 (24, 33)**	5	**-0.16 (-0.21, -0.11)**	338	**-0.006 (-0.008, -0.003)**
Step test (steps)	**47 (28, 66)**	37	**0.09 (0.03, 0.15)**	306	**0.02 (0.01, 0.03)**
Functional reach test (cm)	**50 (14, 85)**	42	**0.15 (0.02, 0.28)**	301	**0.06 (0.03, 0.09)**
Lateral reach test (cm)	**33 (12, 54)**	10	0.20 (-0.05, 0.45)	333	**0.02 (0.01, 0.04)**

Bold denotes statistical significance, $p<0.05$.
Higher values of timed up and go test represent poorer performance whereas higher values of all the other tests represent better performance.
†Adjusted lower limb muscle strength and balance measures were used (Adjusted for age, weight and height, education level and strenuous physical activity).

8.4 Discussion

This is the first study to our knowledge that has investigated both linear associations between LMS and clinical tests of balance in middle-aged women, and the potential for thresholds that could identify women at higher risk of balance problems. Results from linear analyses provide strong evidence that even in middle-aged women, poorer LMS is associated with reduced balance. This supports the concept that prevention of falls by addressing poorer muscle strength could begin far earlier than old age, in an attempt to ameliorate the impacts of age-related losses in muscle strength on balance and ultimately falls and fracture. However, the benefits to falls and fractures may be only likely if the higher strength and balance are maintained. While cut-points were identified, the confidence intervals for these were wide other than for the TUG, and with balance measures other than the ST were driven by a few influential data points. Thus, even though identifying cut-points may be useful to assist screening middle-aged women early to identify women at potentially higher risk of developing impaired balance, our study does not provide evidence to definitively identify appropriate cut-points for this purpose.

Few other studies have examined associations between muscle strength and balance in middle-aged adults [13, 28, 29]. Two are consistent with our findings. One cross-sectional study in 1346 women aged 53 years reported that greater grip strength was associated with better chair rise performance and the ability to balance on one leg with eyes open for 5 seconds[28]. Analysis of baseline data in a pre-post study of a muscle strengthening intervention in 26 middle-aged women (mean age 52.8 (SD 2.4) years) reported that greater maximal isometric bilateral leg extension force was moderately associated with better performance in the test of "10-m walk time" at

baseline (r = -0.6), though no effects on static balance or time of standing on 1 leg or climbing for 10 steps were observed[29]. This may be explained by the fairly good physical condition and moderate muscle strength of study participants before training. This study also demonstrates the potential for strength training to improve balance in middle-aged women as the intervention improved LMS, 10-m walking time (at maximal or normal speed) and dynamic balance tests[29]. In contrast, one small cross-sectional study did not identify any associations between balance and LMS measures in middle-aged adults (n=32 of whom only 9 were female, mean age 56 (SD 4) years) [13], probably due to the very small sample size[13]. This cross-sectional data does not allow causal inferences to be made and longitudinal data in middle-aged women are currently lacking. Longitudinal data in healthy middle-aged men (45-68 years old) has shown that those in the lowest and middle tertile of baseline grip strength were at greater risk of developing functional limitations and disabilities than those in the highest tertile. This suggests that those with greater reserve of muscle strength in midlife can lose more strength before they reach the safety margin of disability[30] and with our data provides some support for a potential strategy of intervening early to improve muscle strength reserves in midlife. Nevertheless, further prospective longitudinal studies in middle-aged women would assist with establishing a causal relationship between LMS and balance in this population.

Interventions targeting LMS are likely to be exercise interventions, as these are effective at improving muscle strength throughout lifespan[31-33]. For example, as mentioned, the study of Holviala et al showed that both LMS and balance could be significantly improved by strength training in middle-aged women[29]. However, this was a pre-post design and these findings need confirmation by well-designed randomised controlled trials. Importantly, whether improving muscle strength and

balance in midlife can reduce the risk of falls in older age remains unknown. Potentially, long-term trials with decades of follow-up would be needed to assess this. Such studies will be logistically difficult to undertake, and it may be that this will need to be inferred from high quality longitudinal observational studies.

Importantly, there is a rapid age-related decline in balance and muscle strength commencing between 45 and 55 years of age[7], suggesting potential benefits of early interventions targeted to improve muscle strength and balance for the prevention of falls and disability in older age. While we identified statistically significant cut-points for associations between LMS and balance tests, these had wide confidence intervals, and were driven by a few influential data points. Sparse data at the low end of LMS may be explained by the relatively younger age and good physical condition of participants, or alternatively by the inability of participants with the lowest LMS to participate in studies such as this. Evidence for threshold values from larger studies is required before being considered for clinical use.

Our study has other limitations. Causality cannot be inferred from our cross-sectional results. For example, physical limitations from impaired balance could adversely influence muscle strength, if people perform less physical activity. Thus even though our results are consistent with limited longitudinal observational [30], and trial data [29, 34], a direct causal relationship needs to be confirmed by well-designed RCTs in younger or middle-aged women. Generalisability of our findings may be limited by the original study's inclusion and exclusion criteria, the predominantly Caucasian nature of the population [35] and the present study being a cross-sectional analysis of data from the 74% of the cohort retained 12 years after the original population-based study[17]. However, our previous comparison of baseline characteristics with the

Tasmanian population at baseline suggested that there were only minor effects of the potential of selection bias towards a healthy cohort [35]. Women who were lost to follow-up were younger, had lower levels of education, and were more likely to be current or past smokers, and less likely to be married or in a de facto relationship compared to those who were retained[27], but the wide spread of education levels at baseline and employment rate at 12 years approximates the overall population figures for these socioeconomic factors and adjustment for potential confounders was performed. Therefore, while this sample may be not fully representative of the Tasmanian population and the generalizability of our study findings to other racial/ethnic populations is uncertain, our findings are likely to be generalisable to healthy Caucasian women in this age range.

In summary, our study shows that in middle-aged women, poorer LMS is associated with reduced balance, while no evidence was found for thresholds of LMS below which there are stronger associations with balance. A useful strategy to improve balance and reduce falls risk in later life may be to intervene to improve muscle strength in middle-age. However, this needs to be confirmed by trials with long term follow-up.

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Appendix 8.A: Publication of "Chapter 8: Lower limb muscle strength and balance in middle-aged women"

Wu F, Callisaya M, Laslett L, Wills K, Zhou Y, Jones G, Winzenberg T. Lower limb muscle strength is associated with poor balance in middle-aged women: linear and nonlinear analyses. Osteoporosis International. 2016:1-8 (in press).

Wu, F., Callisaya, M., Laslett, L. L. et al., (2016). Lower limb muscle strength is associated with poor balance in middle-aged women: linear and nonlinear analyses, Osteoporosis international, 27(7), 2241-2248

Chapter 9: Summary and future directions

Given its substantial public health implications and the poor compliance with drug therapies, the prevention of osteoporosis is of great importance. Although osteoporosis occurs most often in postmenopausal women, the long-term maintenance of peak bone mass by slowing premenopausal bone loss is critical. Several strategies to address this were explored in this thesis.

9.1 Summary of findings

In Chapter 4, by performing an additional 10 years follow-up of a 2-yr osteoporosis education RCT, the first study was able to determine the long-term effects of the feedback of bone density derived relative fracture risk in combination with osteoporosis education on osteoporosis knowledge and self-efficacy. The improvements in osteoporosis knowledge from group education (the OPSMC) and from receiving feedback of high fracture risk previously reported at two years[(1)] did not persist 12 years from baseline. At 12 years, osteoporosis knowledge remained higher than baseline in all intervention groups but the change in osteoporosis over 12 years was similar in both fracture risk groups and both education groups. There were no differences in changes in self-efficacy between intervention groups at either 2 years or at 12 years. This suggests that more frequent intervention is likely to be needed to maintain the additional benefits of feedback of high fracture risk and group education on osteoporosis knowledge in the long-term and that other approaches to improve self-efficacy are required. A more specific education session focusing on improving self-efficacy may be useful, and it should be designed based on the four

main sources that form and affect self-efficacy: personal accomplishment, verbal persuasion, vicarious experience, and physiological or affective states[2].

Chapter 5, examines the long-term effects of the above-mentioned interventions on more clinically important outcomes; that is, BMD and several osteoporosis preventive behaviours (calcium supplement, vitamin D supplement, light and moderate physical activity, cessation of smoking). In the only data of its kind, we demonstrated that feedback of high fracture risk slowed loss of FN BMD, improved the use of calcium and vitamin D supplements and were suggestive of a favourable effect on smoking status. Group education (OPSMC) improved smoking behaviour compared to a leaflet but did not have additional benefits for FN BMD. These findings have implications for osteoporosis prevention in that fracture risk feedback based on BMD could be considered in young women as a strategy to improve long-term bone health and prevent osteoporosis in later life. Importantly, the resulting improvements in behaviours could also be beneficial for other health issues beyond bone. The fact that a relatively simple behavioural intervention was able to produce such long-standing effects is also likely to be influential for the design of interventions for other chronic diseases.

Chapters 6 and 7 report on associations between modifiable risk factors of serum 25(OH)D levels (Chapter 6) and physical activity and time spent sedentary (Chapter 7) on a range of outcomes relevant to musculoskeletal health, namely FN and LS BMD, LMS and measures of static and dynamic balance.

Chapter 6 presents the first report estimating the optimal level of serum 25(OH)D for a range of musculoskeletal outcomes in middle-aged women. This is based on its cross-sectional associations with multiple clinically important musculoskeletal

endpoints, namely BMD, LMS and balance in a population-based cohort of Australian middle-aged women (aged 36-57 years). We identified cut-points for associations between serum 25(OH)D level and most outcomes (range from 29 to 33 nmol/L), below which greater 25(OH)D level was associated with increased BMD and LMS as well as better performance on balance tests, while above which, there were no such associations. These associations suggest that a 25(OH)D level of at least 29 to 33 nmol/L could be required for optimal musculoskeletal health in this population. The current cut-off of 50 nmol/L, as recommended by the Institute of Medicine, may be higher than needed for some outcomes but appears warranted overall. Additionally, these cut-points can assist in designing dose-response intervention trials to determine the definitive optimal vitamin D status.

In Chapter 7, the fourth study assessed independent associations between objectively-measured physical activity and time spent sedentary with musculoskeletal health outcomes (especially BMD) in a population-based sample of middle-aged women. In middle-aged women, greater total physical activity (CPM) and MVPA was associated with better outcomes (BMD, LMS and balance) and sedentary time with poorer musculoskeletal health outcomes (balance). Greater time spent in MVPA contributes to better musculoskeletal health, independent of time spent sedentary but not vice versa, suggesting that increasing time spent in MVPA may be more important than decreasing sedentary time, for musculoskeletal health in middle-aged women. These findings are important for developing interventions to improve habitual physical activity that are targeted at improving musculoskeletal health among women in midlife when an accelerated process of decline in BMD, muscle strength and balance starts.

As both muscle strength and balance begin attenuating in middle age, Chapter 8 explored both linear and nonlinear associations between LMS and balance in a population-based sample of middle-aged women. LMS was associated with poorer performance on a range of balance measures (timed up and go test, step test, functional reach test and lateral reach test) in middle-aged women. There are cut-points of LMS for all balance tests (29-50 kg); however, excepting step test, cut-points did not persist after excluding potentially influential data points. These findings suggest that in middle-aged women, poorer LMS is associated with reduced balance. Therefore, improving muscle strength in middle-age may be a useful strategy to improve balance and reduce falls risk in later life. Middle-aged women with low muscle strength may be an effective target group for future RCTs.

In summary, the findings from this thesis underline the importance of fracture risk feedback based on BMD for improving osteoporosis preventive behaviours and long-term maintenance of BMD in younger women. In addition, we have identified other potentially modifiable factors that could be targeted to improve musculoskeletal health, in middle-aged women, specifically, vitamin D status, MVPA and LMS.

9.2 Future directions

Future directions as suggested by this thesis are to:

1. *Examine whether the findings in this thesis are generalisable to men*

   It should be noted that all these findings in Chapter 9.1 may not be generalisable to men, and similar studies will be needed to confirm this in men. This is important for developing guidelines that cover the whole population.

2. *Determine whether the observed long-term effects of BMD feedback on osteoporosis preventive behaviours and BMD in younger women will reduce fracture risk in later life.*

   This could provide more direct and stronger evidence for considering adding this intervention to the guideline as a strategy for early prevention of fractures in younger women.

3. *Improve the evidence base for identifying optimal levels of serum vitamin D for musculoskeletal health in middle-aged women by:*

   a. *Examining whether the identified cut-points for associations between serum 25(OH)D and musculoskeletal health outcomes in middle-aged women exist in longitudinal studies, and if so,*
   b. *Performing a randomised controlled trial to determine whether supplementation of vitamin D is more beneficial in women with baseline 25(OH)D level below than above the identified cut-points.*

   This could be used to identify a subgroup of individuals whose 25(OH)D level are suboptimal for musculoskeletal health so we can better target supplementation to those most likely to benefit and avoid supplementation in those who are not vitamin D deficient. Furthermore, by accurately determining the magnitude of the effect of supplementation in at-risk individuals, the cost-effectiveness of vitamin D screening and supplementation in the age group can be more accurately assessed. In addition, studies have been conducted mainly in Caucasian populations and estimates for the 25(OH)D threshold may differ across racial groups because of differences in the extent of vitamin D deficiency and the associations between 25(OH)D levels and bone health. Therefore, such studies in non-Caucasian populations are also needed.

Nonetheless, such RCTs of vitamin D supplementation would be challenging as there are potential ethical issues of not supplementing someone who is clearly vitamin D deficient. Rosen and Khosla have argued that women at very high risk should be excluded in a placebo-controlled trial of a new intervention when standard treatments are known to be effective at reducing fracture risk[3], and a history of a fragility fracture of the hip or spine, a very low BMD (T-score<-2.5), or both could be considered as the criteria of identifying high risk women[3]. Therefore, a RCT of vitamin D supplementation aiming to improve BMD, muscle strength and balance might still be feasible in middle-aged women as most of them are not likely to have osteoporosis[4] or fractures of hip[5] or spine[6] and a treatment would not be recommended.

4. *Determine whether greater muscle strength and/or better balance in middle-aged women is associated with reduced risks of falls and fractures in older age.*

   Some physical performance assessments, such as timed up and go test, have been demonstrated to be highly predictive of fracture risk even after accounting for the effects of low BMD and other clinical risk factors for fracture in older women[7]. However, it remains unknown whether these feasible inexpensive performance assessments if performed in younger women could also improve the value of BMD in terms of prediction of fracture risk in older age. This will provide important evidence to support suggestions that prevention of functional limitations, falls and fractures in older age via improving muscle strength and balance should begin in midlife.

5. *Determine whether interventions to improve LMS could help maintain balance in middle-aged women.*

   This could provide stronger evidence to support important role of muscle strength for maintaining balance in middle-aged women. If this could be confirmed, improving muscle strength should be considered in strategies for maintaining balance in midlife, and possibly preventing falls and fractures in later life. The most effective intervention for improving muscle strength might be resistance training, which has been demonstrated in older adults[8]. In addition, supplementation of nutrients, such as vitamin D[9] and protein[10] may also be promising and feasible.

6. *Determine whether promoting MVPA could preserve bone density, muscle strength and balance in middle-aged women by performing a RCT.*

   The observational data of Chapter 7 have shown that MVPA is more important to musculoskeletal health outcomes than light physical activity or sedentary time in middle-aged women, but stronger evidence is required to support implementing programs to improve MVPA for improving musculoskeletal health in middle-aged women. RCT data would provide such evidence. Moderate-to-high intensity progressive resistance training, such as jumping exercises, could be an ideal and feasible exercise modality for improving MVPA in order to improve BMD and muscle strength in premenopausal women[11,12].

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Appendix 1. Questionnaires used in the original 2-yr RCT

A. General Measures

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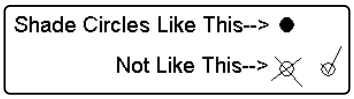
BONE DENSITY STUDY FOR PRE-MENOPAUSAL WOMEN LIVING IN THE HOBART AREA

Instructions for completing questionnaire:

Please write in block letters using a black pen (if possible)

Indicate your response by filling in the circle next to the most appropriate answer or by writing clearly in the boxes or space provided.

Example:



Name: _____

Date of Birth: _____

Todays Date | | / | | / | | | | |

Weight | | | . | | kg

Height | | | cm

1. Sunlight Exposure

What is the average length of time per day that you spend outside (please fill one circle in each section for summer and winter)

	Summer (Dec/Jan/Feb)		Winter (Jun/Jul/Aug)	
a) Weekdays	Less than two hours per day	○	Less than two hours per day	○
	2 - 3 hours per day	○	2 - 3 hours per day	○
	3 - 4 hours per day	○	3 - 4 hours per day	○
	More than four hours per day	○	More than four hours per day	○
	Summer (Dec/Jan/Feb)		**Winter (Jun/Jul/Aug)**	
b) Weekends	Less than two hours per day	○	Less than two hours per day	○
	2 - 3 hours per day	○	2 - 3 hours per day	○
	3 - 4 hours per day	○	3 - 4 hours per day	○
	More than four hours per day	○	More than four hours per day	○

2. Is the main financial provider in your household unemployed or on a pension? **Yes** ○ **No** ○

3. Are you in paid employment?

No ○
Yes (< 20 hours per week) ○
Yes (>20 hours per week) ○

4. What is your present marital relationship?

Single ○
Married, living together ○
Married, separated ○
Unmarried, living together (defacto) ○
Unmarried, not living together ○
Divorced ○

5. Bone Density Results

	BMD	T Score	BMC
Lumbar Spine			
Femoral Neck			
Total Body			

Lean Mass ☐☐☐☐☐.☐☐ Fat Mass ☐☐☐☐☐.☐☐

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6. Leg Muscle Strength Strength #1 ☐☐☐ Strength #2 ☐☐☐

7. Physical work capacity (Endurance Fitness)

If there is no significant increase in the participants heart rate between the second and third minutes of the test then increase the work load to 1.0 Kg and commence readings from there. This is the only time at which a work load of 2.0 Kg will be used.

Minute	Time	Heart Rate	Work Load	Minute	Time	Heart Rate	Work Load
1	☐:☐☐	☐☐☐	0.5	9 / 5	☐:☐☐	☐☐☐	1.5
2	☐:☐☐	☐☐☐	0.5	10 / 6	☐:☐☐	☐☐☐	1.5
3	☐:☐☐	☐☐☐	0.5	11 / 7	☐:☐☐	☐☐☐	1.5
4	☐:☐☐	☐☐☐	0.5	12 / 8	☐:☐☐	☐☐☐	1.5
5 / 1	☐:☐☐	☐☐☐	1.0	9	☐:☐☐	☐☐☐	2.0
6 / 2	☐:☐☐	☐☐☐	1.0	10	☐:☐☐	☐☐☐	2.0
7 / 3	☐:☐☐	☐☐☐	1.0	11	☐:☐☐	☐☐☐	2.0
8 / 4	☐:☐☐	☐☐☐	1.0	12	☐:☐☐	☐☐☐	2.0

Incomplete Bike Test

Equipment malfunction ○
Technique difficulties ○
Refused to continue ○
Elevated pulse rate ○
Physical restrictions ○
Abnormal Heart Rate ○

General Comments

Difficulty in maintaining RPM ○
Erratic RPM ○
Physical Limitations ○

Comments

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B. Calcium Food Frequency Questionnaire

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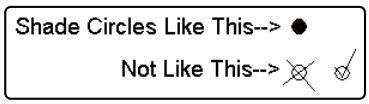
FOOD FREQUENCY QUESTIONNAIRE FOR CALCIUM INTAKE

Instructions for completing questionnaire:

- Please write in block letters using a black pen (if possible)
- Consider your usual dietary habits over the past 12 months.
- Indicate your response by filling in the circle next to the most appropriate answer or by writing clearly in the boxes or space provided.

Example:



Date : / / DOB: / /

First Name :

Surname :

Maiden Name :

Address:

City: Postcode:

Home Phone: Work Phone:

Mobile:

Friend/relative contact details (not living at home address)

Firstname:

Surname:

Relationship Home Phone:

Mobile Phone Work Phone:

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Please record YOUR intake of the following foods:

MILK

1. How much milk in total do you usually use each day for yourself?

None	○	300 to 600 mls	○
Less than 150 mls ..	○	600 mls to 1 litre	○
150 to 300	○	More than one litre ..	○

2. If you eat breakfast cereal how much milk do you usually add?

None ..... ○
1/4 cup ..... ○
1/2 cup ..... ○
1 cup ..... ○
more than one cup .. ○

3. How many cups of tea or coffee with milk do you usually drink each day? ☐☐

4. What type of milk do you usually drink?

No Milk	○	Skim	○
Whole milk	○	Hi-lite	○
Diet lite	○	Physical	○
Light start	○	Soy Milk	○
Form	○	Other	○

CHEESE

5. What type of cheese do you usually eat? Please write each type eg cheddar

How much of the following foods do you eat each DAY?

Food Type	Amount per DAY	Equivalents
EXAMPLE *Wholemeal bread*	*7 slices per day*	*1 slice = 25 g*
6. BREAD Wholemeal bread	☐☐ Slices	1 slice = 25 g
White bread or other	☐☐ Slices	1 slice = 25 g
7. YOGHURT Natural yoghurt	☐☐☐☐ grams	1 small carton = 200 g 1 tablespoon = 30 g
Fruit yoghurt	☐☐☐☐ grams	1 small carton = 200 g 1 tablespoon = 30 g

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How much of the following foods do you eat each WEEK?

Food Type	Amount per WEEK	Equivalents
EXAMPLE *Muesli*	*13 tablespoons per week*	*3 tablespoons = 60 g*
7. CHEESE Hard / tasty cheese	☐☐ Slices	1 slice = 30 g
Soft / Cream / Cottage Cheeses	☐☐☐☐ g	1 small carton = 250g
8. ICECREAM	☐☐☐☐ grams	1 scoop = 50 g
9. EGGS	☐☐ large or ☐☐ medium	1 large = 60 g 1 medium = 45 g
10. FISH Tinned salmon	☐☐☐☐ grams	1/2 cup = 120 g
Tinned sardines	☐☐☐☐ grams	4 - 5 sardines = 60 g
Prawns / Shrimps	☐☐☐☐ grams	3 - 4 pieces = 90 g
Scallops	☐☐☐☐ grams	5 - 6 = 90 g
White Fish	☐☐☐☐ grams	1 medium fillet = 100 g
11. CEREAL FOODS Muesli	☐☐☐☐ grams	3 tablespoons = 60 g
All Bran Cereal	☐☐☐☐ grams	2 tablespoons = 10 g
Sweet biscuits / crackers	☐☐☐ biscuits	1 biscuit = 15 g
Chocolate biscuits	☐☐☐ biscuits	1 biscuit = 15 g
Plain Cake	☐☐☐ slices	1 slice = 40 g
12. FRUITS / VEG. / NUTS Spinach / Silver Beet	☐☐☐☐ grams	1/2 cup = 60 g
Dried Fruits	☐☐☐☐ grams	1 tablespoons = 15 g
Peanuts	☐☐☐☐ grams	18 - 20 nuts = 15 g
13. MISCELLANEOUS Chocolate	☐☐☐☐ grams	4 squares = 20 g
Orange Juice	☐☐ glasses or ☐☐☐☐ mls	1 large glass = 200 ml
14. ALCOHOL White wine	☐☐ glasses or ☐☐☐☐ mls	1 glass = 100 ml
Red wine	☐☐ glasses or ☐☐☐☐ mls	1 glass = 100 ml
Beer	☐☐ glasses or ☐☐ stubbies	1 stubby = 375 mls 1 glass = 200 ml

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15. Do you take any calcium or multivitamin tablets? ○ Yes ○ No

If so please specify type, amount and frequency.

16. Do you take any antacids or indigestion tablets? ○ Yes ○ No

If so please specify type, amount and frequency.

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C. Physical Activity Questionnaire

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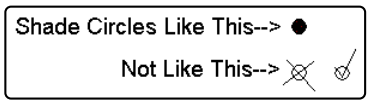
PHYSICAL ACTIVITY QUESTIONNAIRE

Instructions for completing questionnaire:

Please write in block letters using a black pen (if possible)

Indicate your response by filling in the circle next to the most appropriate answer or by writing clearly in the boxes or space provided.

Example:



For purposes of this questionnaire consider your physical activity over the past 12 months.

A. On how many days during the last 14 days did you spent at least 20 minutes doing strenuous exercise?
E.g. bicycling, brisk walking, jogging, aerobics, etc that was severe enough to raise your pulse rate, cause you to breathe faster.

(1) No days ..... ○
(2) 1 to 2 days ..... ○
(3) 3 to 5 days ..... ○
(4) 6 to 8 days ..... ○
(5) 9 or more days ..... ○

B. On how many days during the last 14 days have you spent at least 20 minutes doing light exercise?
E.g. walking, light housework, slow bicycling, etc. Exercise which was not severe enough to cause a pulse rate rising and or breathing increase.

(1) No days ..... ○
(2) 1 to 2 days ..... ○
(3) 3 to 5 days ..... ○
(4) 6 to 8 days ..... ○
(5) 9 or more days ..... ○

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C. During a normal week, how many hours a day do you spend watching T.V. or videos?

(1) No hours a day ..... ○
(2) 1 hour or less a day ..... ○
(3) 2 to 3 hours a day ..... ○
(4) 4 to 5 hours a day ..... ○
(5) 6 or more hours a day ..... ○

D. During the last 12 months, how many team or individual sports activities did you participate in either on a competitive or professional level? E.g. tennis, netball or golf.

(1) No sports or activities ..... ○
(2) 1 sport or activity ..... ○
(3) 2 sports or activities ..... ○
(4) 3 sports or activities ..... ○
(5) 4 or more sports or activities ○

What sports or activities did you participate in?

1. [ ] 2. [ ]
3. [ ] 4. [ ]
5. [ ] 6. [ ]
7. [ ]

E. Please tick off all the sports or activities which you participated in more than 10 times during the last 12 months. Please include team sports.

Aerobics ..... ○
Basketball ..... ○
Netball ..... ○
Volleyball ..... ○
Bicycling ..... ○
Bowling ..... ○
Dancing ..... ○
Gardening ..... ○
Bushwalking ..... ○
Rollerblading ..... ○
Swimming ..... ○
(Laps or water sports like water polo or underwater hockey)

Power walking ..... ○
Jogging ..... ○
Soccer ..... ○
Softball ..... ○
Hockey ..... ○
Tennis ..... ○
Squash ..... ○
Badminton ..... ○
Gym-work weight training . ○
Golf ..... ○

Any other activities or sports which are not mentioned here [ ]
[ ]
[ ]

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D. The Osteoporosis Knowledge Assessment Tool

Questionnaire on Osteoporosis

Reg #: | | | |

Please answer each of the following questions with either True, False or Don't know.

Shade Circles Like This--> ●
Not Like This--> ⊗ ✓

1. Osteoporosis leads to increased risk of bone fractures.	○ True	○ False	○ Don't know
2. Osteoporosis usually causes symptoms (e.g. pain) before fractures occur.	○ True	○ False	○ Don't know
3. Having a higher peak bone mass at the end of childhood gives **no** protection against the development of osteoporosis in later life.	○ True	○ False	○ Don't know
4. Osteoporosis is more common in men.	○ True	○ False	○ Don't know
5. Cigarette smoking can contribute to osteoporosis.	○ True	○ False	○ Don't know
6. White women are at highest risk of fracture as compared to other races.	○ True	○ False	○ Don't know
7. A fall is just as important as low bone strength in causing fractures.	○ True	○ False	○ Don't know
8. By age 80, the majority of women have osteoporosis.	○ True	○ False	○ Don't know
9. From age 50, most women can expect at least one fracture before they die.	○ True	○ False	○ Don't know
10. Any type of physical activity is beneficial for osteoporosis.	○ True	○ False	○ Don't know
11. It is easy to tell whether I am at risk of osteoporosis by my clinical risk factors	○ True	○ False	○ Don't know
12. Family history of osteoporosis and fractures strongly predisposes a person to osteoporosis.	○ True	○ False	○ Don't know
13. An adequate calcium intake can be achieved from two glasses of milk a day.	○ True	○ False	○ Don't know
14. Sardines and broccoli are good sources of calcium for people who cannot take dairy products.	○ True	○ False	○ Don't know
15. Calcium supplements alone can prevent bone loss.	○ True	○ False	○ Don't know
16. Alcohol in moderation has little effect on osteoporosis.	○ True	○ False	○ Don't know
17. A high salt intake is a risk factor for osteoporosis.	○ True	○ False	○ Don't know
18. There is a small amount of bone loss in the ten years following the onset of menopause.	○ True	○ False	○ Don't know
19. Hormone therapy prevents further bone loss at any age after menopause.	○ True	○ False	○ Don't know
20. There are no effective treatments for osteoporosis available in Australia resent.	○ True	○ False	○ Don't know

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E. The Osteoporosis Self-efficacy Scale

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Osteoporosis Self-Efficacy Scale

We are interested in learning how confident you feel about dong the following activities. We all have different experiences, which will make us more or less confident in doing the following things. Thus, there are no right or wrong answers to this questionnaire. It is your opinion that is important. In this questionnaire, EXERCISE means activities such as walking, swimming, golfing, biking and aerobic dancing.

Please fill the circle of the option that best describes your confidence level.

Shade Circles Like This--> ●

Not Like This--> ⊗ ✓

If it were recommended that you do any of the following THIS WEEK, how confident or certain would you be that you could:

1. Begin a new or different exercise program
   ○ Not at all confident ○ Mildly Confident ○ Confident ○ Very Confident
2. Change your exercise habits
   ○ Not at all confident ○ Mildly Confident ○ Confident ○ Very Confident
3. Summon up the effort required to exercise
   ○ Not at all confident ○ Mildly Confident ○ Confident ○ Very Confident
4. Perform exercises even if they are difficult
   ○ Not at all confident ○ Mildly Confident ○ Confident ○ Very Confident
5. Exercise for the appropriate length of time
   ○ Not at all confident ○ Mildly Confident ○ Confident ○ Very Confident
6. Do the type of exercises that you are supposed to do
   ○ Not at all confident ○ Mildly Confident ○ Confident ○ Very Confident
7. Increase your calcium intake
   ○ Not at all confident ○ Mildly Confident ○ Confident ○ Very Confident
8. Change your diet to include more calcium rich foods
   ○ Not at all confident ○ Mildly Confident ○ Confident ○ Very Confident
9. Eat calcium rich foods as often as you are supposed to
   ○ Not at all confident ○ Mildly Confident ○ Confident ○ Very Confident
10. Select appropriate foods to increase your calcium intake
    ○ Not at all confident ○ Mildly Confident ○ Confident ○ Very Confident
11. Stick to a diet which gives an adequate amount of calcium
    ○ Not at all confident ○ Mildly Confident ○ Confident ○ Very Confident
12. Obtain foods that give an adequate amount of calcium
    ○ Not at all confident ○ Mildly Confident ○ Confident ○ Very Confident

Appendix 2. Questionnaires used in the 12 years follow-up of the original 2-yr RCT

Please see Appendix 1 for questionnaires used for Calcium Food Frequency, Physical Activity, Osteoporosis Knowledge and Self-efficacy.

A. General questionnaire

Menzies Research Institute Tasmania

Participant ID: ☐☐☐☐☐

Date: ☐☐ / ☐☐ / ☐☐☐☐

THE LONG TERM MAINTENANCE OF BONE DENSITY IN YOUNGER WOMEN STUDY

General Questionnaire

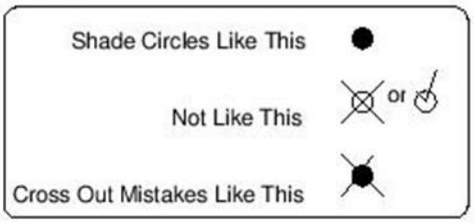
Instructions: Please read carefully

Please answer all questions to the best of your ability (leave blank if unknown).

Your answers will be completely confidential.

Indicate your response by filling in the circle next to the most appropriate answer

Example



Or by writing clearly using the boxes where provided.

Please use BLOCK LETTERS where required e.g H O B A R T ☐☐

Cross out any mistakes & write correct answer just below the relevant boxes

Please use a black or blue pen if possible

If you do not wish to answer any question(s), you are under no obligation to do so. Simply leave the circles or boxes blank for any question you do not wish to answer.

Demographic information

Surname:

Given Names:

Title:

Maiden name:
(if applicable)

Address

Address:

City/Suburb:

State:

Postcode:

Telephone Numbers

Home: -

Work: -

Mobile: -

email:

How long have you lived at this address?

Years

Date of birth: / /

Place of Birth

City/Town:

State/Country:

Demographic (continued)

Is the main financial provider in your household unemployed or on a pension? Yes ○ No ○

Are you in paid employment?

No ○
Yes (< 20 hours per week) ○
Yes (>20 hours per week) ○

What is your present marital relationship?

Single ○
Married, living together ○
Married, separated ○
Unmarried, living together (defacto) ○
Unmarried, not living together ○
Divorced ○

Sunlight Exposure

What is the average length of time per day that you spend outside (please fill one circle in each section for summer and winter)

	Summer (Dec/Jan/Feb)	Winter (Jun/Jul/Aug)
a) Weekdays	Less than two hours per day ○	Less than two hours per day ○
	2 - 3 hours per day ○	2 - 3 hours per day ○
	3 - 4 hours per day ○	3 - 4 hours per day ○
	More than four hours per day ○	More than four hours per day ○
	Summer (Dec/Jan/Feb)	**Winter (Jun/Jul/Aug)**
b) Weekends	Less than two hours per day ○	Less than two hours per day ○
	2 - 3 hours per day ○	2 - 3 hours per day ○
	3 - 4 hours per day ○	3 - 4 hours per day ○
	More than four hours per day ○	More than four hours per day ○

Behaviour

Please indicate if you have changed any of the following behaviours since taking part in the study:

Physical activity	Increased ○	Decreased ○	Stayed the same ○
Calcium intake	Increased ○	Decreased ○	Stayed the same ○
Use of calcium supplements	Increased ○	Decreased ○	Stayed the same ○
Smoking	Increased ○	Decreased ○	Stayed the same ○

Have you ever had a blood test to measure your vitamin D level Yes ○ No ○

If yes, was your vitamin D level Low ○ Normal ○ Don't know ○

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Medications

Please indicate whether you have regularly used calcium supplements in each of the following years. Regular use means taking supplements at least 5 times per week for more than 9 months of the year.

Year	Regularly used?		If used, number of tablets per day		
2000	Yes ○	No ○	One ○	Two ○	More than 2 ○
2001	Yes ○	No ○	One ○	Two ○	More than 2 ○
2002	Yes ○	No ○	One ○	Two ○	More than 2 ○
2003	Yes ○	No ○	One ○	Two ○	More than 2 ○
2004	Yes ○	No ○	One ○	Two ○	More than 2 ○
2005	Yes ○	No ○	One ○	Two ○	More than 2 ○
2006	Yes ○	No ○	One ○	Two ○	More than 2 ○
2007	Yes ○	No ○	One ○	Two ○	More than 2 ○
2008	Yes ○	No ○	One ○	Two ○	More than 2 ○
2009	Yes ○	No ○	One ○	Two ○	More than 2 ○
2010	Yes ○	No ○	One ○	Two ○	More than 2 ○
2011	Yes ○	No ○	One ○	Two ○	More than 2 ○

Please indicate whether you have regularly taken vitamin D supplements in each of the following years. Regular use means taking supplements at least 5 times per week for more than 9 months of the year.

Year	Regularly used?		If used, dose of Vitamin D taken		Office use only Av. daily dose (IU/day)
2000	Yes ○	No ○		or don't know ○	
2001	Yes ○	No ○		or don't know ○	
2002	Yes ○	No ○		or don't know ○	
2003	Yes ○	No ○		or don't know ○	
2004	Yes ○	No ○		or don't know ○	
2005	Yes ○	No ○		or don't know ○	
2006	Yes ○	No ○		or don't know ○	
2007	Yes ○	No ○		or don't know ○	
2008	Yes ○	No ○		or don't know ○	
2009	Yes ○	No ○		or don't know ○	
2010	Yes ○	No ○		or don't know ○	
2011	Yes ○	No ○		or don't know ○	

List all medication, prescribed by a doctor that you have taken in the last 2 weeks, include dosage and frequency.

Please bring all current prescription medication with you to your interview.

	Medication	Dosage	Frequency
a			
b			
c			
d			
e			
f			
g			
h			
i			

List any other over the counter medication you have taken in the last 2 weeks, include dosage and frequency. eg Panadol, Aspirin, Vitamin or mineral supplements, natural or herbal medications and antihistamines.

	Medication	Dosage	Frequency
a			
b			
c			
d			
e			
f			
g			
h			
i			

Smoking

The following questions relate to smoking.

NOTE : A "regular smoker" is someone who has smoked at least 7 cigarettes, cigars or pipes every week for at least 3 months

Have you ever been a "regular smoker"? Yes ○ No ○

If "NO" go to the next section of this questionnaire.

At what age did you first become a "regular smoker"? ☐☐ Years

Are you currently a "regular smoker"? Yes ○ No ○

If No", how old were you when you last gave up being a ☐☐ Years of Age

Have there been any times of at least 6 months or more when you stopped smoking "regularly" but then smoked again afterwards? Yes ○ No ○

If "YES" please indicate how old you were and for how long you stopped.
If this happened more than once record each time.

	Age	Period of time you did not smoke	
		Years	Months
Example	25	01	09
1st time			
2nd time			
3rd time			
4th time			
5th time			

How many cigarettes, pipes or cigars DO/DID you smoke daily on week days?

- 1 - 5 ○
- 6 - 15 ○
- 16 - 25 ○
- 26 - 35 ○
- 36 - 45 ○
- 46 or more ○

How many cigarettes, pipes or cigars DO/DID you smoke daily on weekends?

- 1 - 5 ○
- 6 - 15 ○
- 16 - 25 ○
- 26 - 35 ○
- 36 - 45 ○
- 46 or more ○

What type of tobacco DO/DID you usually smoke?

- Cigarettes - tailor made ○
- Cigarettes - roll your own ○
- Pipes ○
- Cigars ○

Reproductive History Questions

1 What age were you when your periods started? ☐☐ Years

2 Between 20 -40 years of age, how many menstrual periods did you USUALLY have in a year?

NOTE: EXCLUDE times when you were
a. Pregnant or breast feeding.
b. Taking the oral contraceptive pill

11 or more O
between 6 and 10 O
less than 6 O
None O

If none please state the reason ☐

3 Have you ever been pregnant? Yes O No O

If "No", proceed to question 7

4 Enter the appropriate number for each of the following

A. How many times have you been pregnant? ☐☐
B. How many times have you had a miscarriage /termination? ☐☐
C. How many times have you given birth to a child (live or stillborn)? ☐☐

5 The next two questions are about breast feeding.

Have you ever breast fed? Yes O No O

If "No", proceed to question 7

6 How many children have you breast fed? (Include only those children that you have fed for more than one month) ☐☐ Number

7. Have you ever used the oral contraceptive pill? Yes O No O

If "No" proceed to question 9

8 How many years in TOTAL have you ever taken the oral contraceptive pill?

Never O
Less than one year O
1 - 4 years O
5 - 10 years O
11 - 20 years O
More than 20 years O

9. Have you gone through menopause ("Change of Life")
- Yes ○
- No ○
- Don't know ○
- Currently going through menopause ○

10 Have your periods NOW stopped for more than 12 months?
- Yes ○
- No ○
- Never had a period ○

If "Yes" age when periods stopped ☐☐ Years

If "No" go to question 13

If "Never" go to question 12

11 Why did your periods stop?
- Menopause ○
- Hysterectomy ○
- Don't know ○
- Other ○

If Other please specify ☐

12 Have you had a hysterectomy? Yes ○ No ○

If "Yes" age when had hysterectomy ☐☐ Years

13 Have you ever had an operation to remove both ovaries?
- Yes ○
- No ○
- Don't know ○

If "No" or don't know go to question 15

14 Age when ovary/ovaries removed?

One or first ovary ☐☐ Years

Both or second ovary ☐☐ Years

15 Are you currently on hormone replacement therapy (HRT)? Yes ○ No ○

16 For how many years in TOTAL have you ever used hormone replacement therapy?
- Never used ○
- Less than one year ○
- 1 - 4 years ○
- 5 - 10 years ○
- More than 10 years ○

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Fracture/Broken Bone Information

Do you know of anyone in your family who has osteoporosis? Yes O No O

Do you know of anyone in your family who has had a fracture / broken bone? Yes O No O

Have you ever had a fracture/broken bone Yes O No O

If 'No', you have finished this questionnaire. If 'Yes' please provide the details requested below.

Information on first fracture/broken bone

How old was were you at the time of this first fracture/broken bone ☐☐ years ☐☐ months

Which bone was involved (Please indicate left or right)

Was this fracture confirmed by x-ray Yes O No O

Was this fracture obtained by falling Yes O No O

If 'Yes', was the fall from a height of less than 3 metres (ie 0.5 - 3 metres) Yes O No O

What happened (give details)

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Information on second fracture/broken bone

If you have had 1 or none broken bones or fractures you have finished this questionnaire

How old was were you at the time of this second fracture/broken bone ☐☐ years ☐☐ months

Which bone was involved (Please indicate left or right)

Was this fracture confirmed by x-ray Yes ○ No ○

Was this fracture obtained by falling Yes ○ No ○

If 'Yes', was the fall from a height of less than 3 metres (ie 0.5 - 3 metres) Yes ○ No ○

What happened (give details)

If you have had more than two fractures or broken bones please give details below

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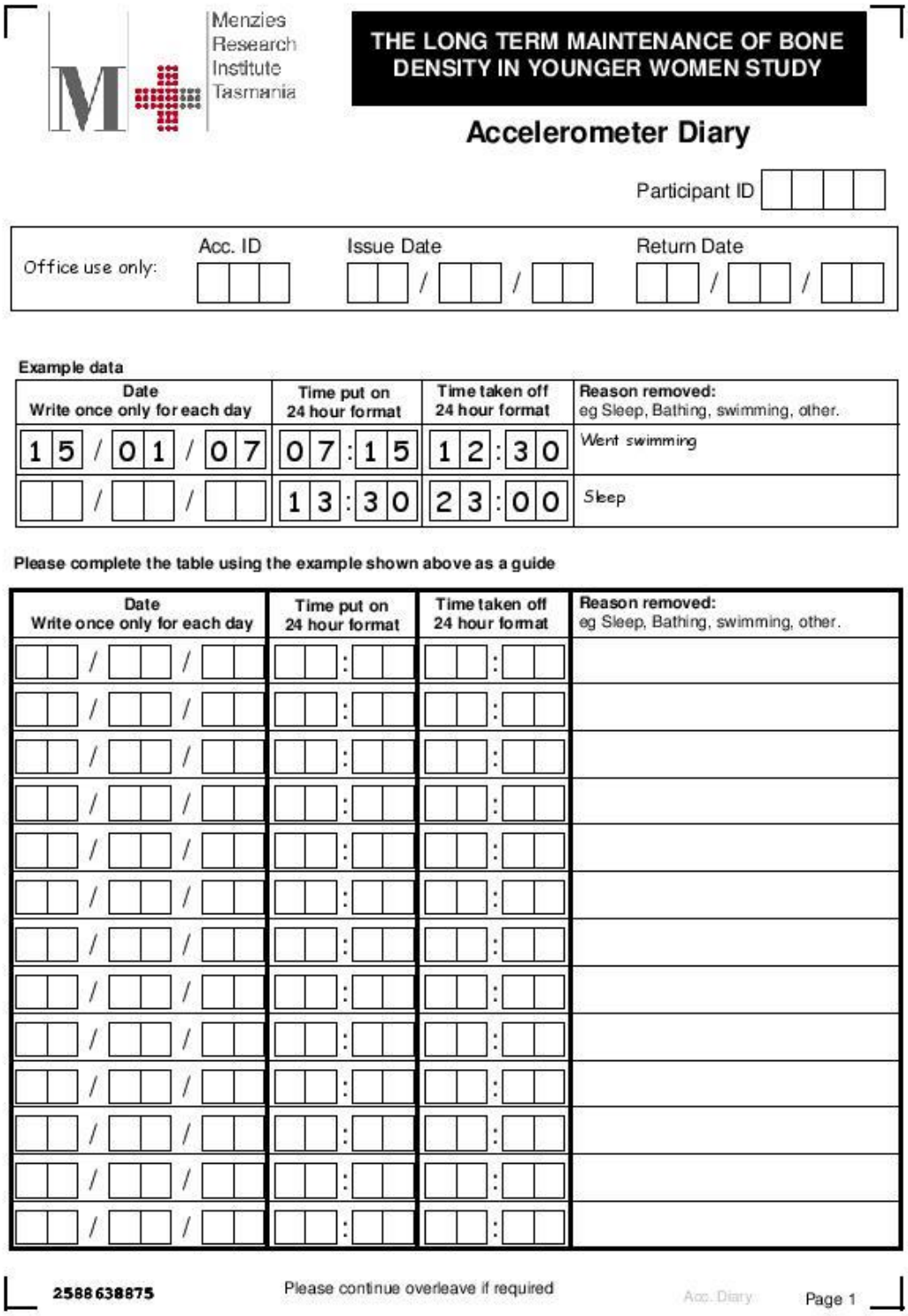
B. Accelerometer Diary



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THE LONG TERM MAINTENANCE OF BONE DENSITY IN YOUNGER WOMEN STUDY

Accelerometer Diary

Participant ID

Office use only:	Acc. ID	Issue Date	Return Date
		/ /	/ /

Example data

Date Write once only for each day	Time put on 24 hour format	Time taken off 24 hour format	Reason removed: eg Sleep, Bathing, swimming, other.
15 / 01 / 07	07 : 15	12 : 30	Went swimming
/ /	13 : 30	23 : 00	Sleep

Please complete the table using the example shown above as a guide

Date Write once only for each day	Time put on 24 hour format	Time taken off 24 hour format	Reason removed: eg Sleep, Bathing, swimming, other.
/ /	:	:	
/ /	:	:	
/ /	:	:	
/ /	:	:	
/ /	:	:	
/ /	:	:	
/ /	:	:	
/ /	:	:	
/ /	:	:	
/ /	:	:	
/ /	:	:	
/ /	:	:	
/ /	:	:	

2588638875 Please continue overleave if required Acc. Diary Page 1

Date	Time put on 24 hour format	Time taken off 24 hour format	Reason removed: eg Sleep, Bathing, swimming, other.
/ /	:	:	
/ /	:	:	
/ /	:	:	
/ /	:	:	
/ /	:	:	
/ /	:	:	
/ /	:	:	
/ /	:	:	
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/ /	:	:	

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C. Clinic questionnaire

Menzies Research Institute Tasmania

Participant ID: ☐☐☐☐☐

Date: ☐☐ / ☐☐ / ☐☐☐☐

THE LONG TERM MAINTENANCE OF BONE DENSITY IN YOUNGER WOMEN STUDY

Clinic Questionnaire

Instructions: Please read carefully

Please answer all questions to the best of your ability (leave blank if unknown).

Your answers will be completely confidential.

Indicate your response by filling in the circle next to the most appropriate answer

Example

Shade Circles Like This	●
Not Like This	⊗ or ⊘
Cross Out Mistakes Like This	⊗

Or by writing clearly using the boxes where provided.

Please use BLOCK LETTERS where required e.g H O B A R T ☐ ☐

Cross out any mistakes & write correct answer just below the relevant boxes

Please use a black or blue pen if possible

If you do not wish to answer any question(s), you are under no obligation to do so. Simply leave the circles or boxes blank for any question you do not wish to answer.

Bone Density

Research Assistant Initials ☐☐☐

Bone density test completed Yes ○ No ○

Date ☐☐ / ☐☐ / ☐☐☐☐

Same as Inteview date (no requirement to fill this date field) ○

If no bone density test taken please state reason

Refusal ○
Physical restrictions ○
Severe breathing ○
> 130 kg ○
other (please specify) ○ ☐

Results given to participant on day of test? Yes ○ No ○

If **No** please record date results sent to participant ☐☐ / ☐☐ / ☐☐☐☐

Questionnaires completed

General questionnaire	Yes ○	No ○
Physical activity	Yes ○	No ○
Calcium intake	Yes ○	No ○
Osteoporosis knowledge	Yes ○	No ○
Self-efficacy scale	Yes ○	No ○
ACCV Food Frequency Questionaire	Yes ○	No ○

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Blood Test

Blood taken — Yes ○ No ○

Date Taken — ☐☐ / ☐☐ / ☐☐☐☐

Time — ☐☐ : ☐☐

Reason for Incomplete blood sample
- Refusal ○
- Patient felt faint ○
- Difficulty finding vein ○
- Vein Collapsed ○

Blood Followup
- New Appointment Made ○
- Arranged to have blood taken elsewhere ○

Comment ☐

Urine sample

Date urine sample collected — ☐☐ / ☐☐ / ☐☐☐☐

What time was urine passed in the morning (24hr time) — ☐☐ : ☐☐

What time did you last pass urine last night (24hr time) — ☐☐ : ☐☐

Comments: ☐

Height & Weight

Height — ☐☐☐ . ☐ cm

Stadiometer Type
- Leicester ○
- Other (please specify) ○ ☐

Stadiometer Number ☐

Weight — ☐☐☐ . ☐ kg

Scales Type
- Heine ○
- Other (please specify) ○ ☐

Scales Number ☐

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Silicon casts

Cast taken of

Left Hand ○
Right Hand ○
Both Hands ○
Not Done (please specify why not) ○ [ ]

Balance

Timed up and go test

Test 1 [ ][ ]
Test 2 [ ][ ]

Step test

	Left	Right
	[ ][ ]	[ ][ ]

Functional Reach test

	Left	Right
Test 1	[ ][ ].[ ][ ]	[ ][ ].[ ][ ]
Test 2	[ ][ ].[ ][ ]	[ ][ ].[ ][ ]
Test 3	[ ][ ].[ ][ ]	[ ][ ].[ ][ ]

Lateral Reach test

	Left	Right
Test 1	[ ][ ].[ ][ ]	[ ][ ].[ ][ ]
Test 2	[ ][ ].[ ][ ]	[ ][ ].[ ][ ]
Test 3	[ ][ ].[ ][ ]	[ ][ ].[ ][ ]

Lower limb muscle strength

Leg Muscle Strength

Strength #1 [ ][ ][ ] Strength #2 [ ][ ][ ]

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Physical work capacity (Endurance Fitness)

If there is no significant increase in the participants heart rate between the second and third minutes of the test then increase the work load to 1.0 Kg and commence readings from there. This is the only time at which a work load of 2.0 Kg will be used.

Minute	Time	Heart	Work Load	Minute	Time	Heart	Work Load
1	:		0.5	9 / 5	:		1.5
2	:		0.5	10 / 6	:		1.5
3	:		0.5	11 / 7	:		1.5
4	:		0.5	12 / 8	:		1.5
5 / 1	:		1.0	9	:		2.0
6 / 2	:		1.0	10	:		2.0
7 / 3	:		1.0	11	:		2.0
8 / 4	:		1.0	12	:		2.0

Incomplete Bike Test

- Equipment malfunction ○
- Technique difficulties ○
- Refused to continue ○
- Elevated pulse rate ○
- Physical restrictions ○
- Abnormal Heart Rate ○

General Comments

- Difficulty in maintaining RPM ○
- Erratic RPM ○
- Physical Limitations ○

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Appendix 3. Other publications during candidature

Wu F, Laslett LL, Zhang Q. Threshold effects of vitamin D status on bone health in Chinese adolescents with low calcium intake. J Clin Endocrinol Metab. **2015**: 100(12):4481-9.

Zhou Y, Zhu G, Charlesworth JC, Simpson S Jr., Rubicz R, Göring H, Patsopoulos NA, Laverty C, **Wu FT**, Henders A, Ellis JJ, van der Mei I, Montgomery GW, Blangero J, Curran JE, Johnson MP, Martin NG, Nyholt DR, Taylor BV. **2016**. Genetic loci for Epstein-Barr virus nuclear antigen-1 are associated with risk of multiple sclerosis. Accepted for publication in Multiple Sclerosis Journal in January 2016.